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CONTENTS: TABLE DES MATIÈRES: INHALT:

J. G. Vermaat and *G. J. van der Bie*, On the occurrence of copper in tropical soils 257

K. Dorph-Petersen and *F. Steenbjerg*, Investigations of the effect of fertilizers containing sodium 283

H. L. Jensen, Periodical variations in nitrogen fixation by *Azotobacter* 301

P. Boischot, M. Coppenet et *J. Hebert*, Fixation de l'acide phosphorique sur le calcaire des sols 311

F. C. Gerretsen, Manganese in relation to photosynthesis . 323

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ON THE OCCURRENCE OF COPPER IN TROPICAL SOILS *)

by J. G. VERMAAT and G. J. VAN DER BIE **)

I. Introduction.
II. Preliminary investigation.
III. Description of the soil samples investigated and provisional classification according to soil type.
IV. Analytical methods used for the determination of copper, organic matter and "matière noire" in the soil.
V. Presentation of analytical data.
VI. Discussion of the results:
a) Influence of soil type on the occurrence of copper.
b) Influence of organic matter.
c) Influence of "matière noire".
VII. Summary

I. *Introduction.* In order to obtain rubbers, both crude and vulcanized, with a high degree of resistance to ageing it has been found that the copper content should not exceed 1,0 mg% (= 0,001%) Cu with reference to the rubberphase [1]).

During an investigation into the copper content of *Hevea* latex of various origins it was found that the copper content sometimes exceeds this critical value. If such a latex was exported or should serve as the raw material for the production of a special rubber, it would very adversely effect the stability of the resulting grades of rubber.

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With regard to the origin of this copper content the following questions arise:

1. Where does this copper come from?
2. If this is known, how does the copper get into the latex?

The first problem to solve therefore is the source of the copper. As possible sources of origin we have the following:

a. The copper is already present in the original soil.

b. The copper is introduced into the soil together with fertilizers.

c. The copper finds its way onto the plant, carried by insecticides or fungicides, and is then absorbed by the plant either directly or via the soil.

d. It is deposited with rain upon the plant or in the soil.

We need not consider the possible presence of copper in the seed since, ultimately, this involves the question of the chicken and the egg.

With regard to these various alternatives the following warrant further consideration:

Among the fertilizers concerned, potassium salts, particularly sulphate and muriate of potash, contain traces of Cu[10]). Various phosphatic fertilizers, like certain natural phosphates and superphosphates derived from them, also contain minute quantities of copper.

The following figures, quoted by Young[10]), give some idea of the amounts involved:

Potassium salts	0–1 mg% Cu (= 0,000–0,001%)
Natural phosphates (Tennessee) .	0–3 ,, ,,
Superphosphates.	0–8 ,, ,,
Ammophos	0 ,, ,,

Some idea as to the amount of copper in organic manures (boengkils *) etc.) may be obtained from the following figures also quoted by Young.

Cotton seed meal	traces
Ricinus boengkil	9 mg%
Screed guano	8 mg%

Thus organic fertilizers contain a good deal more Cu than do inorganic fertilizers. Local composts however show the highest figures. Sjollema (cited by Mulder[5])) reports contents of 20–40

*) Boengkil = residual oil cake.

mg% (fresh weight). A V.A.M. compost from Wijster (Drente) contained 10 mg% (dry weight) according to an analysis made by the State Agricultural Exp. Station in Maastricht.

These figures however do not suggest that these materials are the sources of the copper present in latex.

(*c*) Copper is in fact one of the main constituents of many fungicides. Since however these copper containing materials find no application in *Hevea* cultivation, this possible source may also be ignored.

(*d*) Information concerning the copper content of rain water is rather scanty. In these countries no foundries occur and the atmosphere is therefore not contaminated with copper compounds from flue gases. Consequently this possibility may also be safely discarded.

Considering therefore the various alternatives we must conclude that the possible sources mentioned under *b–d* may be ignored. Thus there remains, as the only possible source, the copper present in the soil.

The following investigations were therefore carried out with the object of establishing the presence of copper in different soils and of ascertaining the way in which it occurs. In the first place however we wish to point out, the desirability of supplementing these investigations later on along the following lines:

a) Influence of the copper content of the soil on that of the latex of a *Hevea* plantation growing on it.

The main questions here will be in what forms the copper occurs and what factors determine the availability of the copper to the plant. The required data may be obtained by analysis of plantation latex and of the corresponding soil samples. These data will enable us to determine which areas, because of their copper content, have to be excluded from latex export.

b) The absorption of copper by the *Hevea* tree and the function of copper in the physiology of *Hevea*.

This subject is outside the scope of the present investigations, but opens up a field for close collaboration between physiologist, soil chemist and latex expert. The research described here is, as a matter of fact, the first result of such collaboration.

II. *Preliminary investigations.* In order to obtain some preliminary information as to

a) the order of magnitude of the amount of copper available to the plant, and

b) differences in the availability of copper in various rubber-soils, the soils were extracted with 3 per cent acetic acid (after Morgan) and the copper in the extract determined in two ways, viz.,

1. by means of Montéqui's drop reaction (see Van Nieuwenburg and Dulfer[7]))

2. by the colorimetric method, using diethyl-dithiocarbamate.

In this way we were in a position to compare both methods. The importance of this lies in the necessity of having at one's disposal a reliable, quick test. If a large number of samples have to be tested later on for copper, this must be done easily and quickly and at a low cost. Montéqui's test has these advantages and at the same time is very sensitive and specific. Iron and manganese, which are always present in soil extracts, will therefore not interfere and no time is wasted in their complete elimination.

Preparation of the acetic acid extract according to MORGAN-VENEMA.

30 grams of air-dry soil are soaked in 6 ml distilled water for 6 hours. 30 ml of a 3 per cent acetic acid solution are then added and after vigorous shaking for one minute the mixture is left for 20 minutes. Again it is vigorously shaken for one minute and then filtered through a dry S & S filter No. 589 II ⌀ 15 cm.

Results of the preliminary investigations

Sixty eight samples from twelve plantations representing various soil types, were analysed. The quantities of Cu varied from 0 to 4 γ Cu per g soil.

The differences between these results and those obtained by the colorimetric method were smaller than the analytical error. Where Fe and Mn were not separated by precipitation however, greater differences were found.

These 68 tropical soil samples show a greater variation in copper content than those of temporate climates. Mulder*) reports 2,5 γ per g air dry soil as being the highest value found by him in Dutch soils.

*) l. c. page 49.

Most of the European researches into the copper content of the soil have been carried out in connection with the so called reclamation disease. It has been shown that peat soils contain substances which fix the copper in such a way as to prevent absorption by the plant. The investigations of M u l d e r and others have shown that this fixation can be ascribed to certain organic compounds. These chemical substances fix the copper in such a way that it is totally unavailable to the plant and is only released upon incineration.

Copper precipitating bacteria may also play a part.

Since we used a very weak extracting agent, the copper extracted by it might be considered to be more or less available to the plant.

It is of interest to know whether some relationship exists between organic matter and copper content. We therefore re-examined the data as far as they were available. These gave the impression that soils poor in organic matter, either originally or owing to erosion of top soil material contain relatively large amounts of copper soluble in 3% acetic acid.

This was the first indication as to the direction in which our investigations had to be pursued.

G u i d i n g p r i n c i p l e s o f t h e i n v e s t i g a t i o n.

We had to take into consideration the fact that the soil might contain a fair amount of copper, but that this is only partially extracted by 3% acetic acid.

Consequently we deiced to go more thouroughly into this problem and began a more extensive investigation on 27 soil samples from our collection, representing five different types of weathering, their organic matter contents ranging from poor to rather rich.

In these samples the amounts of the following items were determined:

a) Cu soluble in the ash (soluble in 2% H_2SO_4),

b) insoluble Cu contained in the ash,

c) Cu soluble in 10% acetic acid *)

d) soluble in 2% H_2SO_4 ($p_H \pm 1.0$) *),

e) organic matter as determined by the dry combustion method of t e r M e u l e n-H e s l i n g a.

f) matière noire.

a) and *b*) give the total amount of Cu present.

*) Determined in the soil as such.

The amount of insoluble copper in the melt represents the copper which is a constituent, though a minor one, of the crystal lattice of minerals and perhaps of some other insoluble compounds, which do not contain silicate.

The copper in the ash and that soluble in 2% H_2SO_4 is derived from both the copper contained in the organic matter and that adsorbed by either inorganic or organic colloids.

In this investigation no distinction is made between copper adsorbed on inorganic and organic colloids.

These figures not only enable us to obtain an insight into the behaviour of copper with the various solvents, but, moreover, allow conclusions to be drawn as to the influence of organic matter and of humus substances on the solubility of copper.

We did not use citric acid for the extraction for fear of complications due to the formation of complexes with copper and iron which would obscure the p_H effect.

The choice of the p_H of the solvents was admittedly rather arbitrary, but was based upon practical experimental reasons.

III. *Description of the soil samples investigated and a provisional classification according to soil type.* Since only the organic matter and humus contents of the soils are taken into consideration, other analytical data are omitted, though recorded in our files. The analytical data relevent to the present investigation are given in Tables I*a* and I*b*.

The main factors which influence the organic matter content of the soil are: temperature, air-water relations and the mineral status of the soil.

The genesis of zonal soil types, at least in its outlines, is nowadays sufficiently well understood. It may be pointed out, however, that this zonality is not restricted only to a horizontal distribution, but a vertical zonality may also exist. M o h r was probably the first to recognise and study this phenomenon, to which M i l n e later gave the name Catena. These catenas play an important role in tropical soil science.

Since man is constantly disturbing natural processes, undisturbed soil types are rather scarce in densely populated areas. Man is constantly interfering with nature by practices such as draining, irrigation, deforestation and cultivation so as to obtain, at least in his own

opinion, the favourable substratum which best suits his requirements. As a result the classification of a number of samples into definite soil types can only be an approximation since there exist numerous transitional types.

1. The lixivial (leached) type. This type is characterized by a permanent downward movement of water as a result of which the upper soil layers are leached out (lixiviated). The substances carried down are partly precipitated or filtered out in the lower horizons giving rise to an accumulation zone, and partly removed via the ground water to rivers and finally to the sea. Leaching impoverishes the soil, but in many tropical regions this process is counterbalanced from time to time, by lava and ashes from active volcanoes. When the temperature is greater than about 20°C, a brown or red lixivium is formed; below this value, however, a bleached lixivium (the so called podzol) results. The formation of these different types at different temperatures is due to the fact that above 20°C the soil is leached with water containing CO_2 resulting from the decomposition of organic matter. Below 20°C leaching is effected by water containing humic substances.

The clay minerals resulting from this type of weathering are of the Kaolinite and related types [3].

According to the degree of weathering one may distinguish between juvenile, virile and senile soils.

Of the samples under investigation, the following represent:

Virile red lixivia

MV 410, 1779, 1781, 1782 (near Tjibadak)
MV 2319, 2321 (near Buitenzorg).

Transitional stage, virile—senile red lixivia

MV 167 (near Bandjar)
MV 197, 216 (near Tjirandjang (Tjandjoer).

Bleached lixivia in the tropics are restricted to altitudes above 1000 m [3]) and are commonly known as "high mountain soils".

Fungi play a predominant role in the mineralizing process at the relatively low temperatutes at which these soils are formed. Leaching therefore takes place with a more or less concentrated humic acid solution. Beneath the fairly extensive humus layer, a light yellow to yellowish green coloured A_2 layer is formed. A humus-iron pan, characteristic of more temporate climates, is generally lacking.

The clay mineral formed is meta halloysite, which is closely relate to kaolinite [3]).

These soils are characterized by a peculiar but very interestin structure, detailed information on which, however, is still lackin Although these podzols are rather acid (down to pH 4.5) they a physically in a good condition and usually well aerated. Because their high organic matter content, these soils are, as a rule, initiall rather rich in N and P.

The following samples belong to this bleached lixivia type

G 1794, 1891, 177	all from Pengalengan	
G 1727, 1904	from G. Patoeah	W. Jav
G 2041	from Soekanegara	
G 1692	from G. Oengaran	M. Jav
G 1783	from G. Wilis	
G 1715, 1961, 1942		
MV 439	from G. Kawi	

Sample G 2041 does not represent the pure bleached lixiviur since the typical structure is absent and its clay content rather high Very probably this is a very much weathered soil.

G 1727 resembles more the true podzol.

2. The intermittently leached type. Since it i represented by only one sample, this type will be discussed ver briefly.

It differs from the lixivium (leaching) of a permeable substratun at a fairly high temperature) in that the downward movement o water is interrupted because of insufficient water during the clearl marked dry periods. The drought is nevertheless not yet so sever that the water tends to move upwards either and the soil does no therefore lose all its moisture. A kind of equilibrium is establishe between rainfall and evaporation, which just prevents the topsoi from drying out completely.

Because of the location of the site and the physical properties o the parent material, this soil type represents a transition towards th subhydric type.

3. The amphibious type. This type occurs in thos areas where the soil is virtually saturated with water during the we season but dries out during the dry season.

The necessary conditions are on the one hand that during the wet season there is a fairly high water table which, depending upon the structure of the soil, falls to more than 0.5–1.5 m below the soil surface during the dry season and on the other hand, a pronounced dry period, during which the rainfall for several months does not exceed 60 mm [6]).

This type will therefore prevail in the dry regions of low lying lands, such as alluvial deposits. The smaples MV 1766, 1768, 1771 and 427 came from areas subjected to this type of weathering. MV 427 represents a type of weathering which is shifting towards the lixivial type because of intensive draining as a result of cultivation. Under such conditions it is readily comprehended that the organic matter content is low and aeration of the soil very poor.

4. The subhydric type. Subhydric weathering takes place when the parent material is weathered under water. During this process, the pH gradually decreases to below pH 4.5. At this pH, sesquioxides become mobile and leach out as ions, though it is also possible that they are washed out as sols with organic matter as protective colloids. Bleaching results, giving rise to the white and greyish hues in the profile. When drained, oxidation takes place and the originally reduced iron compounds are oxidized — in so far as they have not already been leached out — and the colour will change to yellow or yellowish brown according to the amount of iron still present.

These soils vary greatly in their organic matter content. The clay mineral formed is kaolinite with some montmorrilonite [3]).

One of the consequences of leaching under acid conditions is that the soils are as a rule, rather poor in bases and hence provide only a rather poor substrate for vegetation. In the present investigation, this type is represented by sample MV 834 from Danau Salak, S.E. Borneo.

Owing to reclamation and permanent drainage, the type of weathering is shifting from the subhydric to the subaerial type but this change has not as yet been in operation long enough, to deprive this soil of its characteristic properties.

IV. *Analytical methods used for the determination of copper, organic matter and "matière noire" in the soil.* 1) Copper. A method devised by the second author for the testing of rubber samples was

used for the determination of the copper content of the soil samples.

The copper content is determined colorimetrically after elimination of the other metals (especially iron) by precipitation of the copper diethyldithiocarbamate, in an acid medium.

Procedure for the estimation of traces of copper. The procedure given here is that used for rubber samples and was slightly modified when applied to soil samples owing to the high ash content of soil compared with that of raw rubber.

Transfer the clear, slightly acid solution of the ash from 1 g of the sample, to a 15 ml centrifuge tube and fill up with water *) to 10 ml. Add 1 drop of concentrated ammonia and 4 drops of a 50 per cent solution of sodium polysulphide (50 g Na_2S. aq. + 1 g sulphur + 50 ml. water); mix well, add 5 drops of conc. sulphuric acid; and mix again. (20 ml of wash-liquid are now made for each centrifuge tube by adding 2 drops of sulphide solution to 20 of 2 per cent suphuric acid). After the samples have stood for one hour, centrifuge for 2 minutes and pour off the liquid. Allow all the liquid to drain out of the tube adn then add 10 ml of wash-liquid, making sure that the precipitate is finely divided. Centrifuge for 1 minute, remove the lquid and repeat washing. After the liquid has drained out of the tube, add 1 drop of 50 per cent sulphuric acid and 1 drop of nitric acid ($d = 1.2$) and carefully heat the tube above a very small flame until all the sulphur has melted down to a yellowish brown globule. Do not allow any further escape of nitrous vapours. After cooling add 10 ml of 2 per cent. sulphuric acid, separate off all sulphur particles by centrifuging and decant the solution into a small flask containing 9 ml of 2 per cent sulphuric acid + 1 ml of a one per cent solution of gumarabic.

To this add 5 ml of an 0,1% solution of sodium-diethyl-dithiocarbanate and with the aid of a Duboscq colorimeter, compare the colour of the solution as quickly as possible, with a standard solution containing 25 γ Cu (in 20 ml 2% sulphuric acid + 1 ml gum-arabic + 5 ml reagent).

With a fifty-millilitre-sample in the colorimeter one mm of standard solution corresponds with 0,5 γ copper in the sample. Accuracy: 0,5 γ Cu.

The standard solution of copper contains 39.23 mg $CuSO_4$. 5 H_2O to 10 ml. (water + 1 drop of sulphuric acid). This standard solution is diluted 1 : 100 and 2.5 ml of this solution are used for each test. The reagent must be kept in a dark bottle and must be renewed every month.

The blank should contain, at the most, 0,5 γ Cu.

Moisture.

Weigh accurately one gram of the air-dry soil into a porcelain crucible and dry at 105°C to constant weight (2 hours). This gives the moisture content.

*) Only water distilled over glass may be used, also when making the reagents.

A s h.

Moisten the contents of the crucible with nitric acid (d = 1.2) and heat carefully over a small flame until no more vapour is given off then heat for 2 hours at 500°C. in an electric furnace. After cooling in a desicator weigh the crucible again. This gives the ash content.

C o p p e r i n t h e a s h.

Add to the ash 10 drops of nitric acid (d = 1.2) and 20 drops of 50 per cent sulphuric acid and heat for 1 hour on a water bath. Then add 25 ml of 2 per cent sulphuric acid and transfer the contents of the crucible to a 50 ml centrifuge tube, carefully triturating the undissolved residue in a mortar. After centrifuging, decant the clear solution into a glass beaker and evaporate on the water bath down to a volume of 5–7 ml. Transfer the contents of the beaker to a 15 ml centrifuge tube and fill up with water to a volume of 10 ml. Precipitate once; treat the precipitate (without washing it out!) with nitric acid + sulphuric acid, add 10 ml of 2 per cent sulphuric acid and then closely follow the directions given above. This gives the copper in the ash.

I n s o l u b l e c o p p e r i n t h e a s h.

Rinse the residue out of the 50 ml centrifuge tube back into the crucible and evaporate to dryness on the water bath. Mix the contents of the crucible with 10 grams of potassium sulphate in a mortar and return the mixture to the crucible. Add 3 ml of conc. sulphuric acid and evaporate (to fuming) above a small flame, then heat for 2 hours in the electric furnace at 550°C. Dissolve the contents of the crucible in 50 ml of water, transfer to a 50 ml centrifuge tube and precipitate with 10 drops of polysulphide solution plus 10 drops of sulphuric acid. acid. After allowing to stand for one hour, centrifuge and decant the liquid. Treat the residue with 2 drops of nitric acid + 2 drops of sulphuric acid, the centrifuge tube ebing placed *in* the boiling water bath for half an hour. Transfer the contents of the tube to a 15 ml centrifuge tube, fill up with water to 10 ml and then follow the instructions closely. This gives the *insoluble copper in the ash.*

C o p p e r s o l u b l e i n 2% s u l p h u r i c a c i d.

Transfer 5 grams of the air-dry soil to a 200 ml E r l e n m e y e r flask. Add 50 ml of 2 per cent sulphuric acid and let the whole stand for 24 hours (shaking every hour at first). Transfer the contents of the flask to a 50 ml centrifuge tube, centrifuge, decant into glass beaker and evaporate to dryness on the water bath. Add 10 drops of nitric acid (d = 1.2) and 20 drops of sulphuric acid and evaporate to dryness on the water bath. Repeat this treatment once, add 10 ml of water, transfer to a 15 ml centrifuge tube centrifuge and decant into 25 ml glass beaker. Wash the residue in the 15 ml tube once with 10 ml of 2 per cent sulphuric acid and, after centrifuging, decant into the same beaker. Evaporate on the water bath down to a volume of 5 ml, transfer to a 15 ml centrifuge tube and fill up with water to 10 ml. From now on follow instructions as outlined above. This is how th copper soluble in 2 per cent sulphuric acid is found.

C o p p e r s o l u b l e i n 10% a c e t i c a c i d.

Transfer 5 grams of the air-dry soil to a 200 ml E r l e n m e y e r flask·

Add 50 ml of 10 per cent acetic acid and let it stand for 24 hours (shaking it at first every hour). Transfer the contents of the flask to a 50 ml centrifuge tube, centrifuge, decant into a glass beaker and evaporate to dryness on the water bath. Generally the extract contains little iron; the residue can therefore be transferred, together with 10 ml of 2 per cent sulphuric acid, straight way to a 15 ml centrifuge tube and be further treated exactly as the instructions state. (In the rare cases when the acetic acid extract contains appreciable amounts of iron, the same procedure should be followed as with the sulphuric acid extract). The above test gives the copper soluble in 10 per cent, acetic acid.

2. Determination of the Organic Matter in the soil.

The organic matter was estimated from the *C* determined by Ter Meulen's method as used by Spithorst[9]).

3. Determination of the "Matière Noire" in the soil.

Allow 10 grams of soil to stand for 24 hours with 50 ml of 4 per cent NH_4OH in an Erlemeyer flask, shaking it from time to time. Then centrifuge. Decant and treat the residue in the centrifuge tubes with a further 50 ml of NH_4OH (4%), stir vigorously and let it stand for one hour. Centrifuge again. This proecedure to repeated until the freshly added NH_4OH ramains virtually colourless after stirring and centrifuging. Wash out the residue with distilled H_2O containing NH_4OH + NH_4Cl. After mixing, the exctract plus wash-water are evaporated on the water bath down to 150 ml The "matière noire" is precipitated with HCl, centrifuged and the precipitate washed out with distilled water. The precipitate is dried at 105°C in a weighed porcelain crucible, then, after cooling, it is weighed, incinerated and again weighed. The difference is "matière noir".

V. *Presentation of analytical data.* The results of the various analyses are tabulated in tabel I*a* and I*b* together with some calculated ratios, which proved to be useful in the interpretation of these data. In addition to the quantities of copper extracted with dilute acids, the amounts of copper in the incinerated soil and in the bisulphate melt were determined. The following values were calculated:

a) Amounts of copper, extracted by 10% acetic acid, 2% sulphutic acid; and those present in ash and in the melt expressed in percentages of total copper.

b) Amounts of copper extracted by 10% acetic acid and 2% sulphuric acid expressed in percentages of the quantity of copper in ash.

c) Difference between quantity of copper in ash and quantity of

copper soluble in 2% sulphuric acid in mg%, corresponding to the quantity of copper fixed as part of an organic molecule.

d) Difference between organic matter and "matière noire", representing the so called circulating organic matter.

e) Quantity of copper (part of an organic molecule) in mg% per 100 g organic matter.

VI. *Discussion of the results.* The analytical figures give us a fairly accurate idea as to the order of magnitude of the quantities of copper present in tropical soils and the forms in which it occurs. We shall first explain therefore how these analyses may be interpreted.

To begin with, we may distinguish in principle, the following types of copper fixation in a soil.

Character of substance, in which copher occurs.	Copper in atomic form as part of a molecule of a substance (insoluble in dilute acid).	Copper in ionic form. Soluble in dilute acid.
A. Inorganic	Silicate-copper	Exchangeable, adsorbed by inorganic colloids
B. Organic	Part of a molecule	Exchangeable, adsorbed by organic colloids

It is unlikely that ionic copper will ever occur as free ion in the soil, but rather it will always be present in an exchangeable form in the sorption complex of the soil. By comparing the quantity of copper in the melt with that in the ash we can distinguish at once between the inorganically and organically bound atomic copper. The quantity of copper in the melt originates from minerals containing copper either in traces or in larger quantities. The copper content of the ash however, is due both to the adsorbed copper and to the atomic copper bound in organic and inorganic forms. The atomic bound copper in both inorganic and organic forms is not extracted by 10% acetic acid and 2% H_2SO_4, unlike the exchangeable copper, which by extraction is exchanged for the H^+ of the above mentioned acids. With regard to the adsorption of copper in exchangeable form we can cite the following investigations. Reinold [8]) made an extensive investigation into the interchangeability of heavy metals in permutites and clays. It appeared that Cu is readily exchanged *). From this it may be concluded that 2% H_2SO_4

*) l. c. page 82 and 83.

TABLE I*a*

Data and analyses of samples												
Estate	Type of weathering	No. of samples	Depth of samplig in cm	Mois-ture content	Ash %	Organic matter %	"Ma-tière noire" %	mg % Copper in				Total Copper mg %
								10% acetic acid	2% sulphuric acid	Ash	Melt	
Tjisalak	Brown or red lixivial (lateric) virile	M.V. 1779	0—45	11.6	76.8	2.17	—	0.05	0.93	1.10	1.25	2.35
,,		M.V. 1781	0—35	10.8	78.4	3.24	0.15	0.03	0.78	1.35	2.55	3.90
,,		M.V. 1782	35—80	12.3	65.9	1.66	0.12	0.04	1.09	1.35	3.05	4.40
Tjirohani		M.V. 410	0—25	9.4	79.5	2.33	0.04	0.06	0.30	1.65	0.95	2.60
Tjiomas		M.V. 2319	0—20	7.8	81.5	2.90	0.26	0.10	0.79	0.80	1.00	1.80
,,		M.V. 2321	0—20	8.1	80.3	2.75	0.18	0.08	0.76	0.80	1.10	1.90
Bangkeloeng Kolot	virile-senile	M.V. 167	9—12	13.2	73.7	4	0.15	0.22	1.30	2.65	1.25	3.90
Matenggeng.		M.V. 216	30—82	10.6	79.3	2.66	0.12	0.19	0.26	0.30	1.25	1.55
Singadjaja		M.V. 197	0—30	5.9	83.1	2.34	0.06	0.18	0.60	0.70	2.15	2.85
Pasir Malang	Bleached lixivial or podzolic	G. 1794	0—60	13.9	66.9	11.09	1.26	0.18	0.80	2.35	1.00	3.35
Soeka Ati.		G. 1891	0—60	12.3	66.6	14.11	1.72	0.18	0.59	2.35	0.90	3.25
Paal V Wonosari		G. 1777	0—60	17.1	63.4	11.95	0.56	0.13	0.33	2.10	1.25	3.35
Patoeah Wattie		G. 1727	0—60	15.3	59.4	17.25	1.87	0.09	0.34	1.20	1.45	2.65
Tjibitoe		G. 1904	0—60	6.8	61.9	17.66	2.15	0.11	0.49	1.60	1.75	3.35
Balapoelang Soekanegara		G. 2041	0— 60	9.8	66.8	12.05	1.28	0.10	0.88	1.70	1.60	3.30
Medini		G. 1692	0—60	15.5	58.9	18.77	3.62	0.06	0.23	3.35	1.30	4.65
Panggoeng Sari		G. 1783	0—60	8.5	75.5	10.25	2.69	0.06	0.68	2.15	1.00	3.15
Gabes		G. 1715	0—60	8.6	73.8	13.33	2.53	0.04	0.45	3.10	1.00	4.10
Gabes		G. 1961	0—60	8.6	64.3	13.84	4.72	0.02	0.39	1.70	1.10	2.80
Wonosari		G. 1942	0—60	9.8	66.8	13.68	4.04	0.05	0.31	3.50	1.00	4.50
Madoe Ardjo		M.V. 439	0—35	7.6	76.8	11.76	4.26	0.03	0.38	1.35	1.55	2.90
Bandjarsari	Intermittent	M.V. 1056	0—10	11.2	80.6	2.85	—	0.07	0.36	0.45	1.35	1.80
Kawoeng	Amphibious	M.V. 1766	30	10.4	82.4	2.16	0.20	0.10	0.92	1.10	2.60	3.70
Kawoeng		M V 1768	30	9 6	82 8	2.41	0.30	0.07	0.87	1.10	2.65	3.75
Kawoeng		M.V. 1771	80	10.5	94.3	0.88	—	0.14	0.97	1.00	2.45	3.45
Soekamandi		M.V. 427	0—20	7.2	83.3	4.53	0.29	0.02	0.61	0.85	3.00	3.85
Danau Salak	Subhydric	M.V. 834	0—19	2.2	94.1	2.08	0.12	0.02	0.03	0.15	0.55	0.70

All figures in columns 6 to 13 on a dry matter basis.

TABLE 1*b*

Calculations based on data of table I*a*

Estate	Type of weathering	No. of samples	Copper as % of total copper in				Copper as % of copper in ash in		Organic matter minus "matière noire"	mg %Cu in ash − mg% Cu in 2% H_2SO_4	mg Cu, part of an organic molecule per 100 g organic matter
			10% acetic acid	2% sulphuric acid	ash	melt	10% acetic acid	2% sulphuric acid			
Tjisalak	Brown or red hydrous (lateritic) virile	M.V. 1779	2.1	39.6	46.9	53.1	4.5	84.5	2.17	0.17	7.8
,,		M.V. 1781	0.8	20.0	34.5	65.5	2.2	57.8	3.07	0.47	14.5
,,		M.V. 1782	0.9	24.8	30.5	69.5	3.0	80.9	1.54	0.26	15.7
Tjirohani		M.V. 410	2.2	11.6	63.5	36.5	3.6	18.2	2.29	1.35	55
Tjiomas		M.V. 2319	5.6	43.9	44.4	55.6	12.5	98.8	2.64	0.01	0.3
,,		M.V. 2321	4.2	40.0	42.2	57.8	10.0	95.1	2.57	0.04	1.55
Bangkeloeng Kolot	virile-senile	M.V. 197	5.7	33.3	67.9	32.1	8.3	49.0	3.85	1.35	33.8
Matenggeng		M.V. 216	12.5	16.8	19.4	80.6	63.5	86.6	2.54	0.04	1.5
Singadjaja		M.V. 197	6.3	21.1	24.2	75.7	25.7	85.8	2.28	0.10	4.3
Pasir Malang	Bleached hydrous or podzolic	G. 1794	5.4	23.9	70.2	29.8	7.7	34.0	9.85	1.55	14.0
Soeka Atti		G. 1891	5.9	18.2	72.3	27.7	7.7	25.1	12.39	1.76	12.5
Paal V Wonosari		G. 1777	3.9	9.9	62.7	37.3	6.2	15.7	11.39	1.77	14.8
Patoeah Wattie		G. 1727	2.5	9.4	45.3	54.7	7.5	28.3	12.38	0.86	5.0
Tjibitoe		G. 1904	3.3	14.6	47.8	52.2	6.9	30.6	15.51	1.11	6.3
Balapoelang Soekanegara		G. 2041	0.3	25.2	51.5	48.5	5.9	51.8	10.77	0.87	7.2
Medini		G. 1692	1.3	5.0	72.0	28.0	1.7	6.9	15.15	3.12	16.6
Panggoeng Sari		G. 1783	1.9	21.6	68.2	31.8	2.8	31.6	7.56	1.47	14.3
Gabes		G. 1715	1.0	11.0	75.6	24.4	1.3	14.5	10.80	2.65	19.9
,,		G. 1961	0.7	13.9	60.7	39.3	1.2	23.0	9.12	1.31	9.5
Wonosari		G. 1942	1.1	6.9	77.7	22.3	1.4	8.8	9.64	3.19	23.3
Madoe Ardjo		M.V. 439	1.0	13.1	46.5	53.5	2.2	28.0	7.50	0.97	8.2
Bandjarsari	Intermittent	M.V. 1056	3.9	20.0	25.0	75.0	15.5	80.0	2.35	0.09	3.2
Kawoeng	Amphibious	M.V. 1766	2.7	24.9	29.7	70.3	9.1	83.7	1.98	0.18	8.3
Kawoeng		M.V. 1768	1.9	23.2	29.3	70.7	6.4	79.1	2.11	0.23	9.5
Kawoeng		M.V. 1771	4.1	28.1	29.0	71.0	14.0	97	0.88	0.03	3.4
Soekamandi		M.V. 427	0.5	15.9	22.1	77.9	2.3	71.7	4.24	0.23	5.1
Danau Salak	Subhydric	M.V. 834	2.9	4.3	21.4	78.6	13.3	20.0	1.96	0.12	5.8

Circulating organic matter = organic matter — "matière noire".

extracts all the exchangeable Cu bound to inorganic colloids, such as clay.

Something is known also about the exchangeability of copper adsorbed by organic colloids M u l d e r *) reports in his thesis that by means of a percolating technique he saturated with $CuSO_4$ two sandy soils, that is soil rather rich in organic but poor in inorganic sorption complex. After being washed out with distilled water, the soil was percolated with 1/20 N HCl. Practically all exchangeable Cu was exchanged by H^+. Consequently we may conclude that copper exchangeably adsorbed by organic colloids will be exchanged quantitatively by 2% H_2SO_4.

On the basis of our analytical data alone however, it is not possible to distinguish between copper ezchangeably bound to inorganic and to organic colloids. Nevertheless it is possible to get an idea about these relationships when the figures are studied together with other data from these soils (see *a*, *b* and *c*).

Although the number of soils examined was relatively small, we divided the copper contents of these 27 samples into five classes, differing by 1 milligram per cent Cu in an ascending scale.

TABLE II

Frequency of Copper content of some Tropical Soils

Content \ Classes	0–1 mg% Cu	1–2 mg% Cu	2–3 mg% Cu	3–4 mg% Cu	4–5 mg% Cu
Total copper	1	4	5	13	4
Copper in ash	8	11	5	3	
Copper in melt	8	12	6	1	

It should be noted that contents of 1.0, 2.0, 3.0, etc. were included in the classes 0–1, 1–2, 2–3, etc. respectively.

We see from this table that nearly half (48%) of the soils examined contain a total copper content of 3–4 milligrams per cent, 63% of the soils contain more than 0.003% and 81% more than 0.002%. The majority of the soils contain less than 0.003% copper, both in the ash and in the melt.

The only research we know of into the copper content of Java soils dates from the early nineties and was carried out by J. G. K r a m e r s of the Oost Java Experimental Station at Pasuruan [4]).

*) l.c. page 64.

Kramers extracted the soil with strong hydrochloric acid and found percentages of the order of 3–12 mg% CuO (which is 2.4–9.6 mg% Cu), recording as a very high percentage, 20 mg% CuO or 16 mg% Cu *).

Seeing that Kramers' analyses were made more than fifty years ago, it need not surprise us to find that the upper limit found by him is higher than ours. His lower values however, are of the same order as those we have found. Probably the analytical method he employed (colorimetric with H_2S) is responsible for the discrepancies, a surmise which is strengthened by the fact that Kramers found contents of roughly 8 mg% Cu †) in soil from the botanical gardens at Buitenzorg (probably Salak soil), whereas we found roughly 2 mg% Cu in the Tjiomas experimental garden (Salak soil).

In an endeavour to track down any relationship which may exist between the amounts of copper found and the properties of the soil, we shall begin by classifying the soils investigated into soil types, because, as explained in Par. III, this may show us what properties have a bearing upon our problem.

a. Influence of the soil type upon the occurrence of copper. The soils of which samples were investigated belong to the lixivial, intermittent, amphibious and subhydric types of weathering.

The various percentages classified within these types give us the following picture.

TABLE III

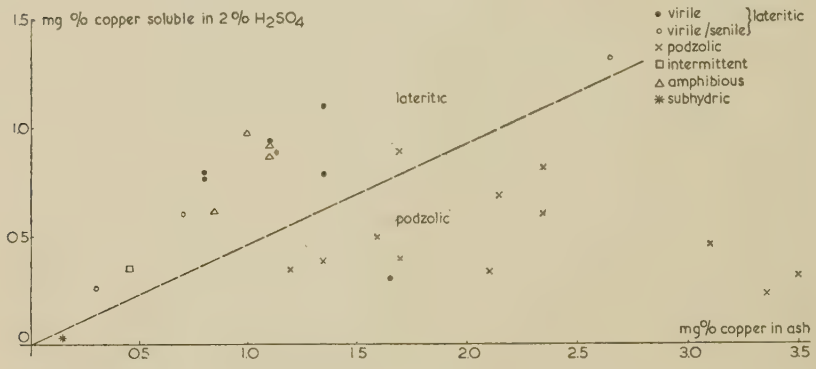
Frequency of Copper content of some Tropical Soil Types

Classes / Weathering	Copper Total					Copper in ash					Copper in melt				
	0-1 mg% Cu	1-2 mg% Cu	2-3 mg% Cu	3-4 mg% Cu	4-5 mg% Cu	0-1 mg% Cu	1-2 mg% Cu	2-3 mg% Cu	3-4 mg% Cu	4-5 mg% Cu	0-1 mg% Cu	1-2 mg% Cu	2-3 mg% Cu	3-4 mg% Cu	4-5 mg% Cu
	Number of samples examined														
wn and red vial . . .		3	3	2	1	4	4	1			2	4	2	1	
ached vial . . .			3	6	3		5	4	3		5	7			
rmittent .		1				1						1			
phibious .				4		2	2						4		
hydric . .						1					1				

*) l.c. pages 44 and 45.

†) l. c. pages 49 and 50.

The lixivial Types of Weathering. As far as the lixivia are concerned, there appears to be a marked difference between the two types in their behaviour both as to the total copper and to that in the ash, in the melt and soluble in 2% H_2SO_4.



Graph. 1. Exchangeable copper.

From a comparison of the copper contents of individual samples of both categories, it is possible to infer the following:

1. Brown and red lixivia have, both absolutely and relatively, more copper bound in the inorganic atomic form than have bleached lixivia (mountain soils).

2. The brown and red lixivia contain a larger proportion of the ionically bound copper in an exchangeable adsorptive form than do the bleached lixivia.

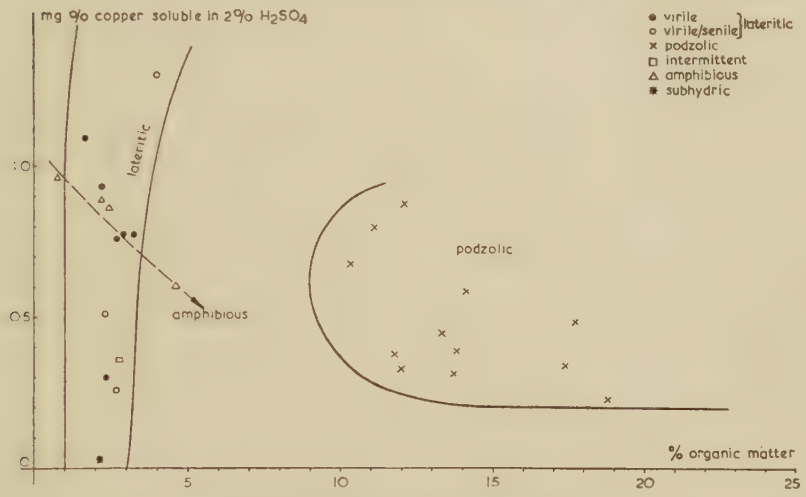
3. The brown and red lixivia have a smaller proportion of the copper in an organic atomic form than the bleached lixivia.

Now in what ways are these differences related to the properties of the two categories of soil?

As explained above, the two types are distinct as to the temperature at which the parent material is weathered, viz. whether or not this happens in the presence of organic matter. The mountain soils are all characterized by a higher organic matter level than the brown and red lixivia. Whereas the latter contain on an average 3%, the brown lixivia from 5–6% (in the most favourable cases there is occasionally a maximum of even 9%), the bleached lixivia contain from 9–20%, depending upon the altitude of the site.

Another distinct point of difference between the soils of the two

types is that brown and red lixivially weathered soils contain a large to very large clay fraction (0,5 μ–2 μ), a moderate loam fraction (2 μ–250 μ) and a very small sand fraction (0,25–2 mm) or practically none at all. The mountain soils, on the other hand, have a small to



Graph. 2. Exchangeable copper and organic matter.

moderate sand fraction, a fairly large loam fraction and a small clay fraction, increasing with the degree of weathering.

With this in mind then, we are able to say that parallels are discernible between the manner in which the copper is bound, on the one hand, and the properties of the soil, on the other:

1. The larger percentage of organo-chemically bound copper in the mountain soils goes hand in hand with the higher percentage of organic matter contained in them.

2. The mountain soils are distinguished by a far lower clay content (inorganic adsorption complex) than the brown and red lixivia, which seems to go together with a smaller quantity of exchangeable adsorptively bound copper.

It should follow from this that the major part of the copper adsorbed in the exchangeable form is adsorbed by clay and not by organic colloids. We shall return to this under *b* and *c*.

The Intermittent Type of Weathering. We have only one sample of this type, but, applying the same reasoning as that adopted for the lixivia, we are able to observe that:

1. With a low total Cu content the major part of the copper must be present in the inorganic atomic bound form (Cu in melt), with only a small fraction in the organic atomic form.

2. The fact that, upon extraction of the soil with 2 per cent H_2SO_4, eighty per cent of the quantity of copper found in the ash dissolves, points to a mainly exchangeable adsorptive binding.

This sample exhibits the same characteristics as those belonging to the brown and red lixivia. This corroborates the contention as to the genetic relationship of the two types. Further investigation will have to show however, whether the observations made on this single sample are borne out by other representatives of this type.

The Amphibious Type of Weathering. The soils of Soekamandi and Kawoeng belong to the amphibious type. The top-soil samples of Kawoeng have the same total copper content; the subsoil contains a little less. The top-soil sample of Soekamandi contains virtually as much copper as the two Kawoeng top-soils.

In this soil only a relatively small amount of copper is organically bound in atomic form and the greater part of the copper present in the ash is bound exchangeably to the adsorption complex.

Hence, in its behaviour towards copper, this type closely resembles the brown and red lixivially weathered soils.

The Subhydric Type of Weathering. Here again we had only one sample for our work.

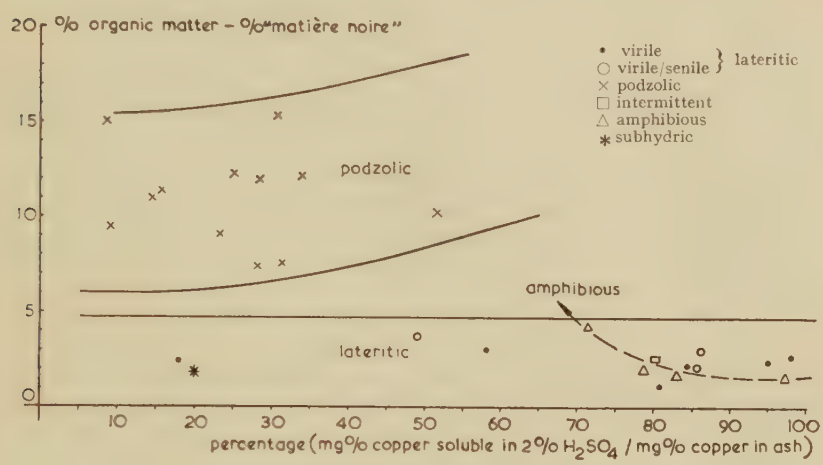
With regard to its behaviour towards copper, this type occupies an intermediate position between the brown and red lixivia (high percentage of copper in the melt) and the mountain soils (high percentage of copper bound in an organic molecule).

The relationships discerned may be conveniently set out as follows:

Types of weathering	Copper content		
	Silicate	Part of an organic molecule	Exchangeably adsorptive
1. Lixivial weathering			
a. brown and red soils	high	low	high
b. mountain soils	low	high	low
2. Intermittent weathering. . . .	high	low	high
3. Amphibious weathering . . .	high	low	high
4. Subhydric weathering	high	high	low

b. Influence of organic matter. It has already been pointed out that the two factors which control the availability

of copper in the soil were organic matter and content of colloidal material.



Graph. 3. Ecxhangeable copper and circulating organic matter.

In the organic matter the copper occurs in two ways:

a. either as constituent of an organic molecule, in which form we referred to it as atomic copper (because it is electrically neutral) or,

b. as an ion, exchangeably and adsorptively bound by colloidal material.

As far as the organic matter itself is concerned we may distinguish between the humus, which is more or less stable, and the readily decomposable matter.

The humus portion in our opinion most closely corresponds to the "matière noire".

The organic matter, which is readily decomposed in nature, either photochemically or by microorganism, is constantly being added to the soil by all kind of organic waste. It may be called organic matter in circulation. In our data it corresponds to the difference between organic matter as determined according to Ter Meulen-Heslinga, and the "matière noire".

We can calculate the amount of copper bound in organic molecules in mg per g organic matter.

These quantities are rather small.

Looking over the corresponding data and graphs we arrive at the following conclusions:

1. The atomic copper increases with the total amount of organic matter (humus and circulating).

2. Generally the bleached lixivia (mountain soils) contain more atomic copper than does any one of the other types.

3. Bleached lixivia contain a smaller proportion of the ash copper exchangeably adsorbed in the circulating organic matter than do the others.

4. In all probability the amount of atomic copper per g organic matter is larger in the bleached lixivia than in the others, although,

5. the absolute quantities of ionic copper are of the same order of magnitude.

TABLE IV

Frequency of quantities of atomic copper in mg per 100 g organic matter					
Class / Soiltype	0,0-0,05 mg Cu	0,05-0,100 mg Cu	0,100-0,150 mg Cu	0,150-0,200 mg Cu	0,200 mg Cu
	Number of samples				
Brown and red lixivium	4	1	1	1	2
Bleached lixivium	1	4	4	2	1
Intermittent weathering	1		—	—	
Amphibious weathering	1		—	—	—
Subhydric weathering	1		—		—

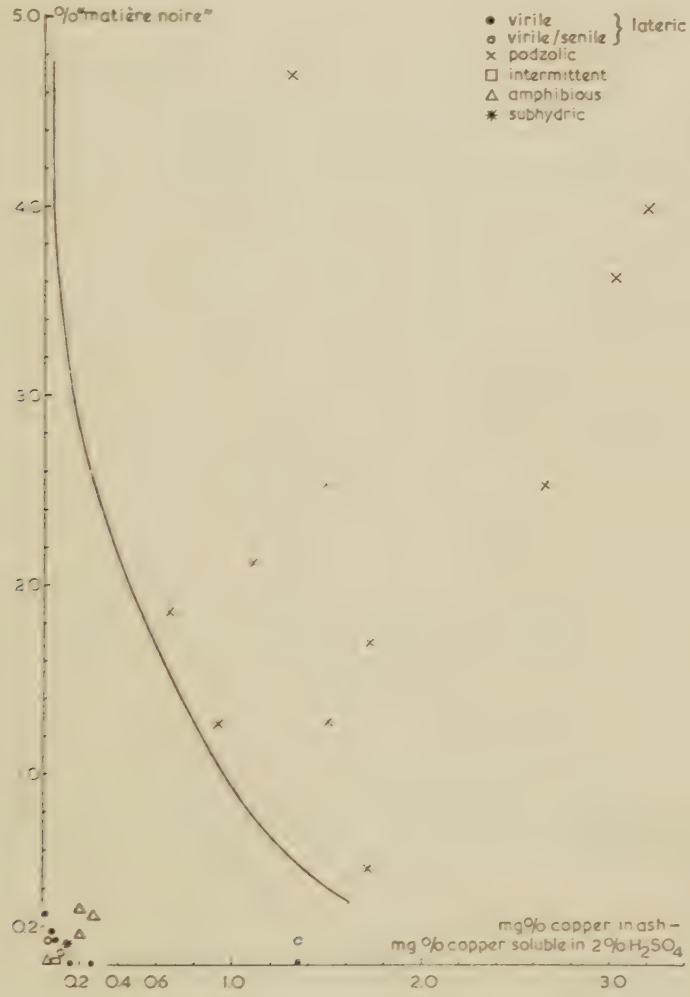
These differences in behaviour between the bleached lixivia (podzolic) and the other weathering types with respect to copper may be explained in the following way:

We fail to see why the parent material of the bleached lixivial soils should originally contain a quantity of copper different from that of the other types.

During the process of soil formation however, and also later on, the bleached lixivia, owing to much slower mineralization, come to contain a higher organic matter content and consequently are in a position to fix more atomic copper set free by the weathering process, than are the other types.

Although the absolute amounts of ionic copper in both types are of the same magnitude, a relatively larger quantity of the ash copper is adsorbed by the brown and red lixivia and other types than by the bleached lixivia. This means that in bleached lixivia a larger part of the copper is fixed in molecules of the circulating organic matter, and, as in the brown and red lixivia, only very little of this is present.

all copper that is not adsorbed to organic or inorganic colloids is washed out and lost.



Graph. 4. Fixed copper and humus.

This would furnish us with a practical technique when we wish to diminish the quantity of available copper in rubber soils, which belong, for the greater part, to the brown and red lixivia. By increasing the quantity of circulating organic matter through mulching we

would fix more copper in organic molecules and thus depress the amount of available copper.

Amphibious soils should require less organic material to obtain the same result.

c. Influence of "matière noire". Apart from fixation in the circulating organic matter we may ask whether the "matière noire" can fix copper in its molecules.

To this end we inspect graph 4. From this one obtains the impression that copper is only fixed when its content has reached a certain limiting value. Below this value all organically fixed copper is in the circulating organic matter.

The interpretation of this might run as follows:

both the micro- and macroflora fix a certain amount of copper per unit organic matter and as long as that level is not reached, none of it gets into the "matière noire".

Above that level, however, virtually all of it is fixed in the "matière noire".

Since at present we have no practical means of incrasing the content of "matière noire" in a soil at will, this has no significance for the time being.

Finally we wish to conclude with the remark that this investigation was carried out during the Japanese occupation of Java with only very limited facilities as far as techniques and choice of soil samples are concerned.

Although conscious of its shortcomings we decided to publish the results now, since in all probability it will be some time before this research is continued and completed in a more satisfactorily way than was possible at the time it was carried out.

Summary

This investigation was induced by the occasional occurrence of injurious amounts of copper in latex.

With regard to the possible sources of origin of the copper in the soil we have:

a. The copper is originally present in the soil.

b. It is introduced into the soil with fertilizers.

c. It is carried by insecticides or fungicides onto the plant and then enters the plant directly or indirectly via the soil.

d. It is carried by rain either to the plant or into the soil. Only the first possibility formed a workeable hypothesis.

The copper occurs in the soil in the following ways:

inorganic - - - → ions / atoms

organic ——————→ ions / atoms

To distinguish between these four different modes of occurrence, the soil was incinerated, the resulting ash digested with HNO_3, treated with H_2SO_4 and the copper present in the ash determined. This figure, the so-called "ash-copper" corresponds to the sum of the copper ions and the copper bound in atom form as constituents of an organic molecule. The insoluble residue of the ash was melted down in bisulphite and the copper was determined again. We consider this copper to be mainly silicate copper (part of the crystal lattice), with a small part of other inorganic origins.

The sum of the copper in the ash and that in melt gives us the total copper.

The air-dry soil was extracted with 2% sulphuric acid. The copper extracted represents, as far as we can see, the copper adsorbed as ions to inorganic and organic colloids. No distinction was made between these two.

The soil was also extracted when 10% acetic acid. We present the data for completeness sake although we did not succeed in interpreting their meaning.

In aiddition to these determinations we analysed the soil for its content of total organic matter and matière noire.

The investigation covered 27 samples in all, representing lixivial, intermittent, amphibious and subhydric types of soil, with regard to the distribution of copper in its various forms we drew up a diagram, from which it appeared that:

a. Brown and red lixivia, intermittent and amphibious types of weathering behave in the same way, as far as adsorption or fixation of the copper are concerned.

b. Bleached lixivial (podzolic) soils behave quite differently.

c. Subhydric soils however, resemble the bleached lixivia with respect to their content of atomic copper, as part of organic compounds and their exchangeabiy adsorbed copper, but with respect to their silicate copper they resemble the red and brown lixivia.

The quantity of copper in ion form was about the same order of magnitude in the brown and red lixivia as well as in the bleached lixivia. We could distinguish between so-called circulating organic matter and more stable material represented by the matière noire.

Soils with a larger quantity of circulating organic matter, such as the bleached lixivia, held more copper fixed than did those containing a smaller quantity. Brown and red lixivia contain a larger part of their ash copper in an adsorbed form than do the belached lixivia.

Soils which contain so much available copper that the latex from *Hevea* trees on those soils is impaired, may be improved by mulching them heavily.

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REFERENCES

1) Bie, G. J. van der, Koperverbindingen in latex en rubber en hun invloed op de duurzaamheid. Thesis, Delft 1948.
2) Hardon, H. J., Factors governing the organic matter and nitrogen contents of tropical soils. Korte Mededeel. Algem. Proefsta. v. d. Landbouw **18** and Landbouw **11**, 517–540 (1935–'36).
3) Hardon, H. J., Chemical and mineralogical investigation of the clay fraction of the principal soil types of the Netherlands Indies. Mededeel. Algem. Proefsta. v. d. Landbouw **37** (1939).
4) Kramers, J. G., Communication No. 49 bij East Java Experimental Station. 42–50 (1893).
5) Mulder, E. G., Over de beteekenis van koper voor de groei van planten en micro-organismen. Thesis, Wageningen 1938.
6) Mohr, E. C. J., De bodem der tropen in het algemeen en die van Nederlandsch Indië in het bijzonder. I (1933) p. 269.
7) Nieuwenburg, C. J. van, and Dulfer, G., A short manual of systematic qualitative analysis by means of modern drop reactions. Amsterdam 1935, p. 16.
8) Renold, A., Kationenumtausch an Permutiten insbesondere an Wasserstoff- und Schwermetall-permutiten. Thesis, Zürich. Kolloid-Beihefte **43**, 1–143 (1936).
9) Spithorst, C., De methodiek van de humusbepaling. Verslag. Landb.-onderzoek. **38**, 65–103 (1932).
10) Young, R. S., Certain rare elements in soils and fertilizers, and their role in plant growth. Cornell Univ. Agr. Exp. Sta. Mem. 174 (1935).

INVESTIGATIONS OF THE EFFECT OF FERTILIZERS CONTAINING SODIUM

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The use of agricultural salt (sodium chloride) as a fertilizer is known from antiquity. Nowadays agricultural salt is used to a fairly large extent in various parts in the world. A study of the effect of agricultural salt was one of the first tasks taken up in field experiments such experiments with agricultural salt being made as early as 1845–46 (reported by Blanck[1])). These field experiments corroborated practical experience that agricultural salt had a promoting effect on the growth of plants, a fact which caused plant physiologists, e.g. Knop in Germany, to postulate that neither sodium nor chloride were substances indispensable to plants, whereas several German authorities on agriculture, among them Liebig, held the view that sodium was necessary to plants. Later this view was changed, and it was discussed whether sodium could wholly or partly replace potassium in plants, a question which was thoroughly treated by Hellriegel and Willfarth[2]) through comprehensive pot experiments.

The effect of agricultural salt and other sodium compounds as fertilizers was later discussed in numerous works. Christensen[3]) has made a survey of publications to 1919, and Blanck[1]) has given a full account of the results of the German experiments and the interpretation of them. Further, Willis[4]) has summarized many investigations on sodium with special reference to the poison effect in connexion with alkali soil problems.

The probem of agricultural salt as a fertilizer for beets is, in Denmark, of extremely small direct importance from the point of view of fertilizing as compared with the importance of the use of ordinary fertilizers. Indirectly, however, the question is of great importance

the quantities of sodium introduced annually in Chilean nitrate, which contains 26–27 per cent sodium, and in ordinary 40 per cent potassium fertilizer, which contains 6—14 per cent sodium, being so great that investigations of their probable effect are of interest.

A. *Field Experiments with Chilean Nitrate, Calcium Nitrate, and Agricultural Salt Made at the Danish Government Experimental Stations 1941–43.* We have results available of 15 experiments with mangolds and fodder sugar beets made on loamy soil (Askov and Lyngby), on sandy soil (Jyndevad, Studsgård, and Hornum), and on marsh (Ribe). The design of the experiments was as follows:

(*a*) Basal dressing (400 kg potassium fertilizer + 400 kg superphosphate per ha).
(*b*) 30 kg N in Chilean nitrate — ab. 200 kg per ha.
(*c*) 60 kg N in ,, ,, — ab. 400 ,, ,, ,,
(*d*) 60 kg N in calcium ,, — ab. 400 ,, ,, ,,
(*e*) 60 kg N in ,, ,, + 400 agricultural salt
(*f*) 60 kg N in Chilean ,, + 400 ,, ,,

Table I is a summary of the results obtained.

TABLE I

Experiments with Chilean nitrate, calcium nitrate, and agricultural salt. Average of the experiments of 1941–43

	Yield and increase in yield, hkg per ha					
	Basal dressing	30 kg N in Chilean nitrate	60 kg N in Chilean nitrate	60 kg N in calcium nitrate	60 kg N in calcium nitrate + 400 kg agr. salt	60 kg N in Chilean nitrate + 400 kg agr. salt
4 experiments with mangolds						
hkg of roots	511	71	160	145	173	171
percentage of dry matter .	14·8	14·6	14·3	14·2	13·7	13·7
hkg of dry matter in root.	75·7	9·4	19·9	17·2	17·7	18·0
hkg of tops	162	18	55	58	60	58
hkg of dry matter in top .	18·9	1·1	4·5	5·2	5·6	5·2
11 experiments with fodder sugar beets						
hkg of roots	339	52	91	77	91	99
percentage of dry matter .	21·1	20·9	20·7	20·7	20·7	20·8
hkg of dry matter in root .	71·5	10·4	17·4	14·6	17·6	19·5
hkg of tops	124	31	73	71	81	82
hkg of dry matter in top .	18·1	3·7	8·6	8·5	8·7	9·0

Agricultural salt applied to mangolds (4 experiments) causes a slight increase in yield in roots whether given with calcium nitrate or Chilean nitrate. By addition of agricultural salt the percentage of dry matter is decreased so much that the increase in yield of dry matter becomes small after application of agricultural salt to calcium nitrate, and negative after applying agricultural salt to Chilean nitrate. Applied to fodder sugar beets (11 experiments) agricultural salt gives a slight increase in yield in roots and tops, both when used with calcium nitrate and when used with Chilean nitrate.

The percentage of dry matter in the roots is not influenced by the application of agricultural salt; hence calcium nitrate + agricultural salt yields as much dry matter as Chilean nitrate, and agricultural salt + Chilean nitrate gives a small increase in yield (2,1 hkg/ha). These results, like the results of other experiments show that, on the whole, agricultural salt applied to calcium nitrate may neutralize the difference in yield otherwise caused by Chilean nitrate and calcium nitrate.

Table I further shows that the application of agricultural salt to low percentage beets, i.e. mangolds and fodder sugar beets with 16–17 per cent dry matter, causes a slight reduction in the contents of dry matter of the roots, whereas this reduction is not observed in the case of high percentage fodder sugar beets and sugar beets. This is particularly evident in the two experiments made at the experimental station of Askov. Here mangolds and fodder sugar beets were grown side by side in the same experiments. The average percentage of dry matter in the two experiments were as follows:

	Mangolds		Fodder sugar beets	
	Hkg of roots	Percentage of dry matter	Hkg of roots	Percentage of dry matter
(*a*) Basal dressing	444	15·2	290	22·5
(*b*) 1 Chilean nitrate	508	15·1	353	22·4
(*c*) 2 ,, ,,	591	14·8	385	22·2
(*d*) 2 calcium nitrate	581	14·7	384	22·3
(*e*) 2 ,, ,, + agricultural salt	605	14·1	392	22·2
(*f*) 2 Chilean nitrate + agricultural salt	611	14·2	396	22·4

In both varieties of beets there is a reduction in the contents of dry matter as the yield of roots increases — unfertilized, $\frac{1}{2}$ Chilean

nitrate, and 1 Chilean nitrate — and, further, there is in mangolds a reduction of the percentage of dry matter in the two plots dressed with agricultural salt, a reduction which does not seem to be due to the slight increase in the yield of roots. Added to fodder sugar beets agricultural salt also gives an increase in the yield of roots, but no particular reduction in the percentage of dry matter. Hence, we may assume that there is a difference between the effects of the agricultural salt on the percentage of dry matter in the varieties of beets.

B. *Pot Experiments with Chilean Nitrate, Calcium Nitrate, Potassium Fertilizer, and Agricultural Salt 1942.* This pot experiment [5]) was planned with the same six treatments as were used in the field experiments at the experimental stations, but so arranged that these six treatments were repeated three times, every time with a different dressing of potassium. The design of the experiments and the chief result in grammes of dry matter per pot appears from Table II.

TABLE II

Pot experiment 1942, grammes of dry matter in roots + tops							
	T_K	Without nitrogen	1 nitrogen Chilean nitrate	2 nitrogen Chilean nitrate	2 nitrogen calcium nitrate	2 nitrogen calcium nitrate + agr. salt	2 nitrogen Chilean nitrate + agric. salt
0 KCl	2·3	20	127	220	105	226	234
1 KCl	11·0	26	197	305	219	217	243
2 KCl	27·0	31	185	267	228	228	233

With the largest quantity of potassium there is doubtless a poison effect (chloride poisoning), but the experiment clearly shows that the difference between the effect of Chilean nitrate and that of calcium nitrate is reduced and that the increase in yield when agricultural salt is applied disappears when a large basal dressing of potassium is applied. This is clear from the following survey:

	Increase in yield to agricultural salt; grammes of dry matter	
	added to calcium nitrate	added to Chilean nitrate
0 potassium fertilizer	121	14
1 ,, ,, ...	—2	—62
2 ,, ,, ...	0	—34

Only in the case of a great deficiency in potassium (2·3 milli-equiv. exchangeable potassium per 2·5 kg air-dried soil) agricultural salt applied to calcium nitrate has caused an increase in yield.

C. *Pot Experiments with Sodium and Potassium in 1943 and 1944.* For a closer investigation of the effect of potassium and sodium applied to beets some pot experiments were made in 1943 and 1944 according to the following plan:

0 sodium fertilizer
1 ,, sulphate
2 ,, ,,
3 ,, chloride.

These four quantities of sodium were each repeated with the following quantities of potassium:

0 potassium
1 ,, sulphate
2 ,, ,,

In all 12 treatments.

The quantities of sodium and potassium in the experimental dressing were per pot:

	1 potassium sulphate	1 sodium sulphate	Sodium chloride
1943	2·57 g K	1·22 g Na	2·50 g Na
1944	2,40 g K	2·47 g Na	2·50 g Na

Thus, in 1943, the treatment with NaCl, corresponded to 2 Na_2SO_4, and hence in what follows will be denoted as 2 NaCl. In 1944 the quantity of Na in NaCl corresponded to 1 Na_2SO_4, and the treatment with sodium chloride will be denoted as 1 NaCl.

As growing medium a mixture of equal parts of sand poor in potassium (Vadgard sand) and loam from undressed plots at the Government Experimental Station at Lyngby was used. Here, as in the preceeding experiments, pots containing20 22 kg soil, height 40 cm, diameter 25 cm, were used. After the first growing season in 1943 the soil was kept in the pots for the growing season in 1944. The contents of water was 60 per cent of the water capacity in the early summer, 80 per cent of the water capacity later in summer. The following quantities of basal dressing were used per pot:

1943	1944
5 g N in calcium nitrate	5 g N in calcium nitrate
12 g secondary calcium phosphate	1 g manganese sulphate
33 g calcium carbonate	0.1 g borax
10 g calcium sulphate	
1 g manganese sulphate	
0·1 g borax	

Each year the experimental crop was fodder sugar beets, and 2 beets were grown per pot. Fig. 1 gives an impression of the development of the roots in the various treatments. The beets were of normal size and form.

In Tables III and IV the individual results of all the experiments are given and an account of various conditions is given in the following summary.

The yield of root and top is increased with increasing addition of potassium and sodium, but the figures of increase in yield for sodium and potassium decrease when respectively the quantity of potassium and the quantity of sodium are increased. This appears clearly from the average figures of the two experiments.

In pot experiments with increasing quantities of two different nutrients, e.g. nitrogen and phosphorus, we generally for a soil with a normal supply of nutrients find the opposite e.g. that an increase of the quantity of a nutrient increases the increase in yield for the other. Hence, it may be assumed that sodium sulphate and potassium sulphate are not two different nutrients, which have different functions in the plants, but that we have to do with substances which, within certain limits, may replace each other. The fact that the sodium effect is not a sulphate effect appears from a comparison with the treatment with sodium chloride (see Table IV*a*).

Boguslawski[6]) in several pot experiments with potassium sulphate and sodium sulphate added to oats has similarly found that the response to sodium decreases with an increasing addition of potassium. The experiments of Holt and Volk[7]) reported below show the same results in several crops. Pot experiments carried out by Heinrich[8]) also indicate that the sodium effect decreases with an increasing addition of potassium.

Pfeiffer et al.[9]) similarly in several pot experiments with barley have obtained a distinct increase in yield with sodium together with a small addition of potassium, but the sodium effect

Fig. 1. One root from each of the 12 treatments. Phot. 1943.

decreases with an increasing addition of potassium. In a large investigation comprising several series of pot experiments Hellriegel and Willfarth [2]) investigated the ratio between the effects of sodium and potassium added to several crops. Even though the experiments, which were made in the 1890's, are affected by certain technical defects, they still show the following principal line. With extremely small quantities of potassium, sodium gives little or no increase in yield.When the application of potassium then increases gradually the inrceases in yield for sodium first increases and then decreases. In one of Boguslawski's experiments we also find that sodium gives no increase in yield with extremely small quantites of potassium. The explanation of this fact must be that sodium cannot replace potassium in all its functions, but that a certain quantity of potassium must necessarily be present for the plants to be able to grow and utilize sodium in certain functions of potassium.

On the basis of the values for the increase in yield in the pot experiments of 1943–1944 we may find an indication of the proportion in which potassium and sodium may replace each other.

Figs. 2 and 3 show the proportion between the supplies of potassium and sodium expressed in milli-equivalents: the increases in yield of the treatments in which only potassium sulphate and sodium sulphate have been added. Graphic interpolation, in Fig. 2 shows that, in the case of small applications, about 3 milli-equivalents of sodium give the same increase in yield as 1 milli-equivalent of potassium. Converted into chloride this will correspond to 235 kg sodium chloride under these conditions being able to replace 100 kg potassium chloride.

The assimilation of potassium and sodium may be calculated from the yield and the crop analyses. The results appear from Tables IV*b* and V.

It appears from Table IV*b* that the assimilation of potassium increases with increasing supply of potassium and, further, that without addition of potassium the application of sodium gives a slight increase in the assimilation of potassium — up to 0·46 g. When potassium sulphate is added, 2·5–5 g per pot, the addition of sodium has no distinct effect on the assimilation of potassium. The slight extra assimilation of potassium effected by the addition of sodium of course will influence the yield, but the increase in yield for sodium cannot be due to this alone, as appears from the ratio between

TABLE III

Results of Pot Experiments 1943

	No potassium				1 K_2SO_4				2 K_2SO_4			
	0 Na	1 Na_2SO_4	2 Na_2SO_4	1 NaCl	0 Na	1 Na_2SO_4	2 Na_2SO_4	1 NaCl	0 Na	1 Na_2SO_4	2 Na_2SO_4	1 NaCl
Yield in g per pot												
Roots	296	432	529	588	724	776	790	818	831	841	914	961
Dry matter in roots	59·7	87·2	109·6	119·2	149·4	161·1	167·5	164·7	178·7	171·9	195·5	203·3
Sugar in roots	34·8	51·3	70·9	77·3	97·1	105·7	110·7	117·0	117·5	119·8	131·9	138·4
Tops	443	539	583	589	614	620	641	658	597	590	653	666
Dry matter in tops	70·7	84·5	89·3	92·8	91·2	90·4	87·4	92·1	90·1	84·3	90·0	96·3
Analyses of the crop, percentage in the crop												
Dry matter in root	20·17	20·18	20·72	20·27	20·63	20·76	21·20	20·13	21·50	20·44	21·39	21·16
Dry matter in top	15.97	15·67	15·32	15·75	14·86	14·58	13·63	13·99	15·10	14·28	13·78	14·46
Sugar in root	11·76	11·88	13·41	13·14	13·41	13·62	14·01	14·30	14·14	14·24	14·43	14·40
Percentage in dry matter												
Potassium, K, in root	0·36	0·30	0·25	0·43	0·64	0·65	0·74	0·69	0·78	0·89	0·93	1·07
Potassium, K, in top	0·47	0·44	0·82	0·49	1·61	1·58	0·96	1·53	3·21	2·74	2·54	2·68
Sodium, Na, in root	0·20	0·27	0.26	0.33	0·11	0·13	0·15	0·16	0·07	0·09	0·10	0·11
Sodium, Na, in top	1·23	2·08	2·53	2·37	1·18	2·04	2·44	2·65	1·29	1·98	2·49	2·50
Soil analyses												
pH value, May 19	7·4	7·5	7·6	7·5	7.5	7·4	7·5	7·5	7·4	7·5	7·6	7·5
pH value, Sept. 24	8·0	8·1	8·1	8·1	8·1	8·0	8·1	8·1	8·1	7·9	8·0	8·2
Phosphorus value, May 19	17	18	18	18	17	19	15	16	17	16	18	18
Phosphorus value, Sept. 24	13	16	13	14	13	16	13	15	15	16	15	15
Potassium value, May 19	1·5	1·6	1·5	1·2	9·7	9·0	8·7	10·0	17·0	16·0	18·0	18·0
Potassium value, Sept. 24	1·6	1·4	1·1	1·1	2·3	2·4	2·2	1·6	3·6	5·0	4·9	5·4

TABLE IV

Results of Pot Experiments 1944

	No potassium				$1\ K_2SO_4$				$2\ K_2SO_4$			
	0 Na	1 Na_2SO_4	2 Na_2SO_4	1 NaCl	0 Na	1 Na_2SO_4	2 Na_2SO_4	1 NaCl	0 Na	1 Na_2SO_4	2 Na_2SO_4	1 NaCl
Yield in g per pot												
Roots	271	476	549	539	601	803	852	805	805	899	985	969
Dry matter in roots	55·6	95·7	115·8	109·0	122·7	162·2	177·1	163·7	163·1	189·6	203·6	203·4
Sugar in roots	34·0	64·9	72·8	69·3	80·2	105·2	117·5	109·8	108·4	128·2	133·8	139·3
Tops	359	568	487	542	496	572	552	580	558	627	602	620
Dry matter in tops	51·9	73·3	64·0	71·9	70·6	74·2	73·7	77·1	75·0	78·6	77·4	83·5
Analyses of the crop, percentage in the crop												
Dry matter in root	20·50	20·11	21·10	20·22	20·42	20·20	20·79	20·33	20·26	21·09	20·67	20·99
Dry matter in top	14·47	12·90	13·15	13·26	14·23	12·98	13·35	13·29	13·44	12·54	12·85	13·47
Sugar in root	12·56	13·64	13·26	12·85	13·35	13·10	13·79	13·64	13·47	14·26	13·58	14·38
Percentage in dry matter												
Potassium, K, in root	0·31	0·21	0·32	0·31	0·72	0·76	0·81	0·78	1·03	0·96	1·06	0·88
Potassium, K, in top	0·50	0·37	0·41	0·39	1·67	1·59	1·43	1·42	2·94	2·39	2·18	2·24
Sodium, Na, in root	0·24	0·32	0·38	0·32	0·13	0·31	0·45	0·29	0·12	0·20	0·33	0·21
Sodium, Na, in top	1·54	2·87	3·70	3·04	1·67	3·20	4·09	3·15	1·67	2·93	4·07	3·14
Soil analyses												
pH value, June 8	7·8	7·8	7·8	7·8	7·9	7·8	7·9	7·8	8·0	7·8	7·9	7·9
pH value, Sept. 30	8·2	8·2	8·2	8·2	8·2	8·2	8·2	8·1	8·2	8·2	8·2	8·2
Phosphorus value, June 8	17	17	16	17	15	15	14	15	16	15	15	14
Phosphorus value, Sept. 30	16	16	16	14	14	13	15	12	13	14	14	14
Potassium value, June 8	1·7	1·9	1·4	1·1	7·5	7·6	7·1	7·3	13·0	16·0	15·0	14·0
Potassium value, Sept. 30	1·3	0·8	1·1	1·4	2·4	2·2	2·7	2·2	2·0	4·5	3·9	3·8

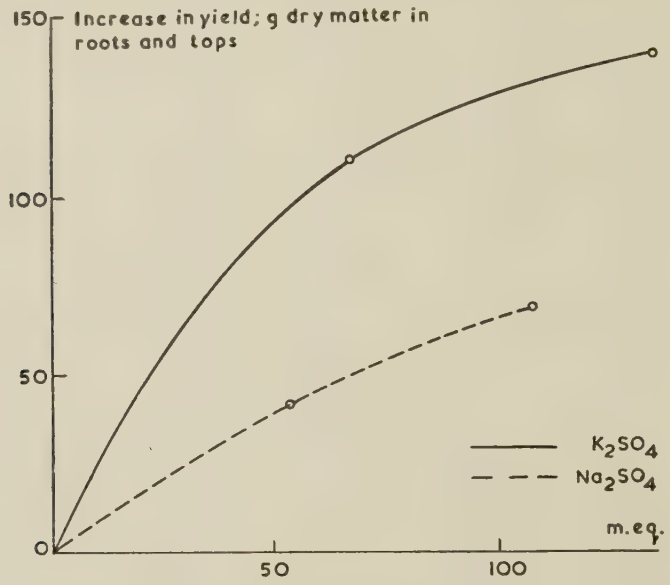

Fig. 2. Pot experiments in 1943. Graph of the increase in yield in proportion to the quantities of potassium and sodium calculated in milli-equivalents.

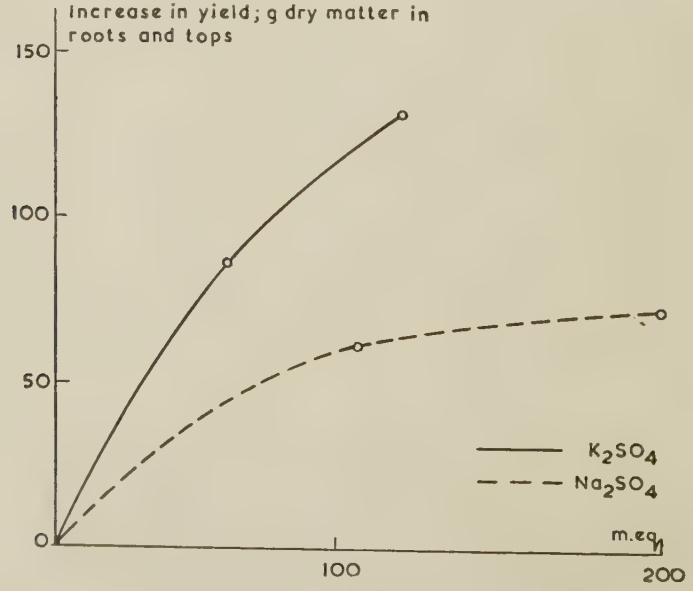

Fig. 3. Pot experiments in 1944. Graph corresponding to Fig. 2.

the assimilation of potassium and the increase in yield in the treatments with potassium.

TABLE IV*a*

Yield and increase in yield in g dry matter in root + top			
	Yield in average of 1943 and 1944		
	0 Na	1 Na_2SO_4	2 Na_2SO_4
0 potassium	119	170	189
1 K_2SO_4	217	244	253
2 K_2SO_4	253	262	283
	Increase in yield for sodium		
0 potassium	—	51	70
1 K_2SO_4	—	27	36
2 K_2SO_4	—	9	30
	Increase in yield for potassium		
0 potassium	—	—	—
1 K_2SO_4	98	74	64
2 K_2SO_4	134	92	94

TABLE IV*b*

The assimilation of potassium, g K per pot				
	Without Na	1 Na_2SO_4	2 Na_2SO_4	NaCl
Pot experiment in 1943				
Without potassium	0·55	0·63	1·01	0·97
1 potassium sulphate	2·42	2·48	2·08	2·55
2 potassium sulphate	4·29	3·84	4·10	4·76
Pot experiments in 1944				
Without potassium	0·43	0·47	0·63	0·62
1 potassium sulphate	2·06	2·41	2·49	2·37
2 potassium sulphate	3·88	3.70	3·85	3·66

Table V shows the assimilation of sodium. The quantity of sodium in the plants increases with the addition of sodium.

Without addition of sodium the application of potassium gives an increasing assimilation of sodium. Further, there was in 1944 a tendency to an increase in the assimilation of sodium due to the addition of potasisum even if sodium was applied.

The cause of the increase in the assimilation of potassium due to the addition of sodium is presumably mainly to be sought in the fact that the quantity of sodium assimilated direct, which gives an increased crop and hence increases the production of carbon dioxide

by the roots of the plants, also increases the exchange and assimilation of potassium. A detailed elucidation of these question, however, has not yet been possible. But the experiments have established that although part of the sodium effect may be due to an increase in the assimilation of potassium, the greater part of the sodium effect must be an effect of assimilated sodium.

TABLE V

The assimilation of sodium, g Na per pot				
	Without Na	1 Na_2SO_4	2 Na_2SO_4	NaCl
Pot experiments in 1943				
Without potassium. . . .	0·99	2·00	2·54	2·59
1 potassium sulphate . . .	1·24	2·05	2·38	2·70
2 potassium sulphate . .	1·29	1·82	2·44	2·63
Pot experiments in 1944				
Without potassium. . . .	0·93	2·41	2·81	2·54
1 potassium sulphate . . .	1·34	2·87	3·81	2·90
2 potassium sulphate . . .	1·45	2·68	3·82	3·05

Further, the relation between the effects of sodium sulphate and sodium chloride may be elucidated by the results of these experiments. It should be kept in mind that in 1943 a quantity of sodium corresponding to 2 Na_2SO_4 was given in the form of sodium chloride, but in 1944 NaCl corresponded to 1 Na_2SO_4. The following Table shows the yield of dry matter and the percentage of dry matter in roots for treatments in which the same quantities of Na have been added as sulphate and chloride.

Yield of dry matter in root + top				
	1943		1944	
	2 Na_2SO_4	2 NaCl	1 Na_2SO_4	1 NaCl
Without potassium. . . .	199	212	169	181
1 K_2SO_4.	255	257	236	241
2 K_2SO_4.	286	300	268	287

There is thus a tendency to Na_2SO_4 giving a smaller yield than NaCl, but because of the variation between the pots with the same treatment this difference is uncertain.

In roots there is a tendency for sodium chloride to give a lower percentage of dry matter than sodium sulphate, but there are several exceptions; for the tops the reverse is the case.

Percentage of dry matter in root				
	1943		1944	
	2 Na_2SO_4	2 NaCl	1 Na_2SO_4	1 NaCl
Without potassium	20·72	20·27	21·10	20·22
1 K_2SO_4	21·20	20·13	20·20	20·33
2 K_2SO_4	21·39	21·16	21·09	20·99

Thus we have not here found any sure difference between the effects of sodium sulphate and sodium chloride, but the latter tends to show the better effect.

D. *Sodium Effect in Crops Other than Beets.* At the Government Experimental Stations in Denmark experiments have been carried out with swedes. The average results of 9 experiments were:

Yield and increase in yield in hkg per ha				
	Roots	Tops	Dry matter in root	Percentage of dry matter in root
Basal dressing	621	116	67·2	10·9
Agricultural salt	23	—1	0·2	10·5
Kainite	58	1	4·3	10·8
Muriate of potash, 40% . .	50	0	5·9	10·9
Muriate of potash, 40% + agricultural salt	56	—3	6·3	10·9

In roots agricultural salt has a slight effect, but as there is a simultaneous decrease in the percentage of dry matter there is no increase in yield of dry matter. It may be stated that at Borris there was in 1915 a high increase in yield with agricultural salt — 165 hkg roots and 14·3 dry matter, whereas the increase in yield at Borris in 1916 was small and at other experimental stations even very small, often negative. In 1915 potassium at Borris showed marked effects, so that this one experiment would seem to indicate that agricultural salt may have a positive effect on swedes when there is a great deficiency in potassium.

In two pot experiments made with sand as growing medium Jacob [10]) found that sodium had no effects where added to swedes. Harmer and Benne [11]) found effects of sodium on turnips grown on peat soil. Likewise Holt and Volk [7]) found an increase in yield with turnips in pot experiments. An average of 10

field experiments in Sweden — Bolin [12]) — gave 58 hkg swedes for 480 kg agricultural salt.

Potatoes. At the Government Experimental Stations experiments with potatoes in 1915–16 gave the following result — the average of 9 experiments:

Yield and increase or decrease in yield. Hkg per ha			
	Tubers	Dry matter	Percentage of dry matter in tubers
Basal dressing	244	57·3	23·6
Agricultural salt	—26	—10·0	21·9
Kainite	8	— 2·9	22·0
Muriate of potash, 40%	21	2·3	23·0
Muriate of potash, 40% + agricultural salt	8	— 3·1	21·9

Added to potatoes agricultural salt gives a decrease in yield of tubers and dry matter, and the addition of salt markedly reduces the contents of dry matter. Still there was an incrase in yield for agricultural salt —17 hkg tubers and 2·6 hkg dry matter — in the experiment made at Borris in 1915 where potassium fertilizer, too, gave a very high increase in yield — 93 hkg tubers and 22·0 hkg dry matter.

Thus, the experiments show that agricultural salt gives either no increase or even a decrease in yield of potatoes and, only in case of extreme deficiency of potassium can it give an increase in yield. Perhaps part of the detrimental effect is to be ascribed to the contents of chloride and not to sodium, for it is known from other experiments that potatoes are sensitive to chlor-ion.

Schulze [13]) and Harmer and Benne [11]) have not found any increase in yield for sodium applied to potatoes. The same is true for some earlier experiments reported by Blanck [1]).

Spring cereals. The Danish Farmers' Associations in 1914–16 made 23 experiments with spring cereals (16 with barley and 2 with dredge-corn) with applications of 100–400 kg agricultural salt — on an average 200 kg. The average results of the experiments were as follows:

	Hkg per ha	
	Grain	Straw
Without agricultural salt	25·8	34·0
200 kg agricultural salt	25·7	35·4

Thus there was no effect of agricultural salt. This yield, which is comparatively high considering the time, would also seem to indicate that the experiments were made on well-manured soil without great deficiency in potassium.

In pot experiments Hellriegel [2]) found effects of sodium added to barley and oats when there was a moderate deficiency in potassium. Effect of agricultural salt added to barley were found by Pfeiffer et al. [9]) and to oats by Holt and Volk [7]) and Boguslawski [6]), whereas Harmer and Benne [11]) did not find any increase in yield for sodium chloride added to barley and oats.

Leguminous plants. Curiously enough there are no reports of Danish experiments on the addition of sodium to clover; which is well-known as a crop with a great consumption of potassium.

In pot experiments made by Hellriegel [2]) there were only small and uncertain effects of sodium added to peas, even at a high deficiency of potassium, whereas Heinrich [8]) found effects of sodium added to peas. Jacob [10]) found effects of sodium added to broad beans in two pot experiments.

Rhodin [14]) in an experiment made in Sweden observed marked effects of sodium chloride added to broad beans, and Holt and Volk [7]) found increase in yield for sodium chloride added to vetches and peas.

Pot experiments with sodium and potassium added to various species of plants made by Holt and Volk [7]) show that, with a few irregularities, the increase in yield decreases for sodium when the addition of potassium increases from 9 18 lbs K_2O to 144 lbs, but this reduction of the increase in yield is not the same in all crops. This experiment shows that all the 7 crops tried may give an increase in yield for sodium.

Summary and Conclusions

1. Pot experiments with increasing quantities of potassium and sodium show that an application of either of these two substances reduces the increase in yield obtained by the other. This means that it is not a question of two different plant nutrients with entirely different functions in the plant, but of two substances with the same — or partly the same — function: in other words, that sodium – within certain limits — can replace potassium. This point of view is also acknowledged by modern plant physiologists such as Boysen-Jensen [16]).

2. The sodium effect is produced by various sodium salts; hence sodium must be the cause, although no doubt the anion is not without effect. Comparisons between calcium nitrate and Chilean nitrate also indicate that sodium is able to increase the amount of plant substance produced, especially when the potassium content of the soil is low [15]).

3. It has been shown, by determining the absorption of potassium and sodium in pot experiments, that the sodium effect is partly due to absorption of potassium on account of the sodium applied. But the greater part of the sodium effect is accounted for by the sodium absorbed by the plants.

4. In field experiments with sodium — mostly agricultural salt — an effect of sodium is generally obtained only when it is given to beets, while in pot experiments and a few field experiments a decisive effect has been noticed for sodium given to other crops. The difference in behaviour of plants to sodium is presumably due to several causes. As sodium only promotes better growth when potassium is deficient, it is to be expected that crops with a great potassium consumption, and therefore more often suffering from a shortage of potassium, are more likely to show an effect of sodium than crops with a small potassium consumption. Beets certainly belong to the greatest potassium consumers among crops, so that the pronounced effect of sodium may to some extent be accounted for by the great potassium consumption of the beet. Amongst other crops with a fairly high potassium consumption potatoes, swedes, clover, and lucerne may be mentioned; but response to sodium has not been obtained in many experiments with these crops. Cereals have only a small potassium consumption and the effect of a supply of sodium together with potassium is often small; hence it is only to be expected that sodium does not produce any increase in yield.

The difference in behaviour of the various plant species towards sodium is presumably, in some measure, due to their differences in potassium consumption.

5. Concerning the practical use of fertilizers containing sodium, the following statement may be made:

The use of Chilean nitrate for beets is to be recommanded on all soils where the occurrence of a plentiful supply of potassium has not already been ascertained, and furthermore when the price per kg of nitrogen in Chilean nitrate and calcium nitrate is about the same. If the price of nitrogen in Chilean nitrate is much higher than in calcium nitrate, the use of Chilean nitrate should be limited to potassium deficient soils.

Under conditions where one is obliged to take a portion of the potassium fertilizer as kainite — with a high sodium content — one should first of all apply the kainite to beets. If potassium can be bought at the same price in concentrated and less concentrated potassium fertilizers, it is always advisable to apply the less concentrated fertilizer — for instance kainite — to beets. Thereby a certain amount of sodium is obtained free of charge and this can produce an increase in the yield of beets.

When agricultural salt is used as a fertilizer, it should be remembered

that it is only of interest in the case of beets. Furthermore, if the beet-fields are manured to such an extent with farmyard and liquid manure that the crop yields 90–100 hkg dry matter per ha, it is doubtful whether it will pay to make use of agricultural salt. It should be remembered that the salt will only produce an increase in yield so long as potassium is deficient.

If one is about to remedy the potassium deficiency of a soil for beet-growing and has the choice of agricultural salt or potassium fertilizer, it should be remembered that salt under ordinary soil conditions produces an increase in yield in the case of beets only; therefore, sodium left behind in the soil will not be utilized by the succeeding crops. Residual potassium, on the other hand, would be utilized. A large part of the sodium taken up by the plants will return to the fields contained in farmyard and liquid manure (dairy farming), but will not be of any value unless beets receive the manure. Potassium, originally contained in the fertilizer and later rotating within the farm, on the other hand, can be assumed to produce a recurrent increase in yield.

Therefore, the economy of using sodium and potassium fertilizers should not be judged by their effect on beets only, because in the case of sodium, the effect will occur once only, whereas potassium can be assumed to have recurrent effect.

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REFERENCES

1) Blanck, G., Die Bedeutung des Natriums für die Pflanze und die sogenannte Kochsalzdüngung. Fühlings Landw. Zg. 1916, 441–463, 508–535.
2) Hellriegel, H. and Willfarth, H., Vegetationsversuche über den Kalibedarf einiger Pflanzen. Arb. deut. Landw. Gesl. Heft 34 (1898).
3) Christensen, Harald R., Forsøg med Kogsalt og Kalisalte. 138 Beretning fra Statens Forsøgsvirksomhed i Plantekultur. Tids. Planteavl **26**, 737–823 (1919).
4) Willis, L. G., Bibliography of references to the literature on the minor elements and their relation to plant and animal nutrition. Third edition 1939. (With 7 Supplements).
5) Steenbjerg, F. and Dorph-Petersen, K., Kvælstof, Kalium og Natrium til Bederoer. Tids. Planteavl. **47**, 668–677 (1943).
6) Boguslawski, Eduard von, Untersuchungen über das Bodenkali sowie seine Aufnahme und Verwertung durch Hafer. Landw. Jahrb. **83**, 711–773 (1936).
7) Holt, M. E. and Volk, N. J., Sodium as a plant nutrient and substitute for potassium. J. Am. Soc. Agron. **37**, 821–827 (1945).
8) Heinrich, Hellmut, Über die Wirkung des Natrons neben dem Kali als Nährstoff der Pflanzen. Z. Pflanzenernäh., Düngung Bodenk. A. **10**, 299–328 (1927/1928).
9) Pfeiffer, Th., Einecke, A., Schneider, W. and Hepner, A., Ver-

suche über die Kali- und Natron-Aufnahme der Pflanzen. Mitt. Landw. Inst. kgl. Univ. Breslau. **3**, 567–613 (1906).

10) Jacob, H., Über die Wirkung des Natrons neben dem Kali als Nährstoff der Pflanzen. Z. Pflanzenernähr., Düngung Bodenk., A **17**, 366–392 (1930).

11) Harmer, P. M. and Benne, E. J., Effects of applying common salt to a muck soil on the yield, composition, and quality of certain vegetable crops and on the composition of the soil producing them. J. A. Soc. Agron. **33**, 952–979 (1941).

12) Bolin, P., Redogörelse för fortsatta jämförande försök med koksalt och kalisalt. Meddelande n:r 94 från Centralanstalten för försöksväsendet på jordbruksområdet. Jordbruksavdelingen nr 27, 1914.

13) Schulze, B., Die Düngewirking des Schwefelsauren Ammoniaks mit Beigabe von Kochsalz. Mitt. deut. Landw. Ges., 452–458 (1910).

14) Rhodin, S., Fältförsöken vid Centralanstaltens för Jordbruksförsök fasta försöksstation Experimentalfältet år 1907. IV. Koksalt till åkerbönor. Kungl. Landtbruks-Akademiens Handlingar och Tidskrift. 1908, 329–332.

15) Iversen, Karsten and Dorph-Petersen, K., Forsøg med forskellige Kvælstofgødninger. 1930–1942. 374. Beretning fra Statens Forsøgsvirksomhed i Plantekultur. Tids. Planteavl **48**, 418–515 (1944).

16) Boysen-Jensen, P., Plantefysiologi. 2. Udgave, København 1943.

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PERIODICAL VARIATIONS IN NITROGEN FIXATION BY *AZOTOBACTER*

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Introduction. Aerobic nitrogen-fixing bacteria (*Azotobacter*) sometimes show erratic growth when tested at different times under otherwise equal conditions. In a preliminary communication, Stapp and Bortels [8]) claimed to have observed a correlation between weather conditions and growth which was strong in good weather under prevailing high barometric pressure, but weak under low-pressure conditions. Bortels [2]) has later discussed the problem in greater detail and has presented a very extensive set of data based on observations continued through several years. He arrived at the conclusion that the growth of *Azotobacter* is vigorous when a high-pressure area approaches the site of the experiment, and remains so while the high-pressure conditions persist; on the other hand the growth becomes weak when a low-pressure is approaching and persisting. The phenomenon was observed in cultures grown with combined as well as with free nitrogen, and seemed also to occur in cultures grown in sterile soil.

No single climatic factor, such as temperature, actual air-pressure, rain or sunshine, or the composition of the atmosphere at different time, seemed to account for the phenomena. As the most likely cause Bortels assumed the existence of an unknown kind of radiation, varying in intensity in accordance with the weather changes, and similar in the nature to the cosmic radiation, but not identical with this. In the majority of his experiments, Bortels used a single strain of *Az. chroococcum*. Growth was estimated by measuring the volume of cell material produced in duplicate cultures during weekly four-day periods at 30°C in 50 ml 2% nitrogen-free mannite solution (not sterilized), and the results were compared with air-pressure

conditions described as "high" or "low", actual figures not being given. Quantitative determinations of nitrogen fixed in a 24-hour period were made in a smaller series of experiments with *Az. agile*. These figures, however, often showed serious disagreements between the duplicates. Bortels expressed the view that *Azotobacter*, owing to the influence of the weather-factor, might be especially active as a nitrogen-fixing agent in soils of geographic regions where high-pressure conditions obtain for long periods, and that this might explain the apparently favourable results of "bacterization" of seeds of non-leguminous plants with *Azotobacter* in Russia, which results so far have not been corroborated elsewhere (cf., for instance, Allison *et al.* [1]) in U.S.A. and Fåhraeus *et al.* [6]) in Sweden).

In later publications, Bortels [3]) [4]) [5]) has extended the weather factor principle to apply to the growth of other microorganisms and to the relationship between parasites and hosts in epidemiological phenomena. Apart from a communication by Hansen and Schwartz [7]), who report a similar relation between weather conditions and the sensitivity of lactic acid streptococci to ultra-short electric waves, the problem does not appear to have been studied by other investigators. In view of the both theoretical and practical importance of the problem, an attempt has been made to reproduce the results of Bortels in a number of tests with *Azotobacter*.

Experimental. Two strains of *Az. chroococcum* were used in the present experiments which were conducted during 16 months, from May 1948 to September 1949. One strain (*A*) was isolated from soil from the grounds of the University of Sydney, Australia, and had a high nitrogen-fixing power. The other (*D*), of moderate vigour, was isolated from a garden soil from Roskilde, Denmark. Duplicate cultures were grown in 25 ml medium in 250-cc Pyrex glass Erlenmeyer flasks. The medium had the following composition: glucose (pure anhydrous) 20.00 g, K_2HPO_4 1.00 g, $CaCO_3$ 2.00 g, $MgSO_4$ $7H_2O$ 0.50 g, NaCl 0.50 g, $FeSO_4$ 0.050 g, Na_2MoO_4 0.005 g, distilled water 1000 ml and was sterilized by autoclaving for 20 min. at 115°C. Inoculum was taken from 2-day cultures on agar medium corresponding to the solution. Cultures were incubated for exactly 120 hours at 25°C and were analyzed for nitrogen by the Kjeldahl method: digestion with 6 ml conc. H_2SO_4, 2 g K_2SO_4, and 0.2 g

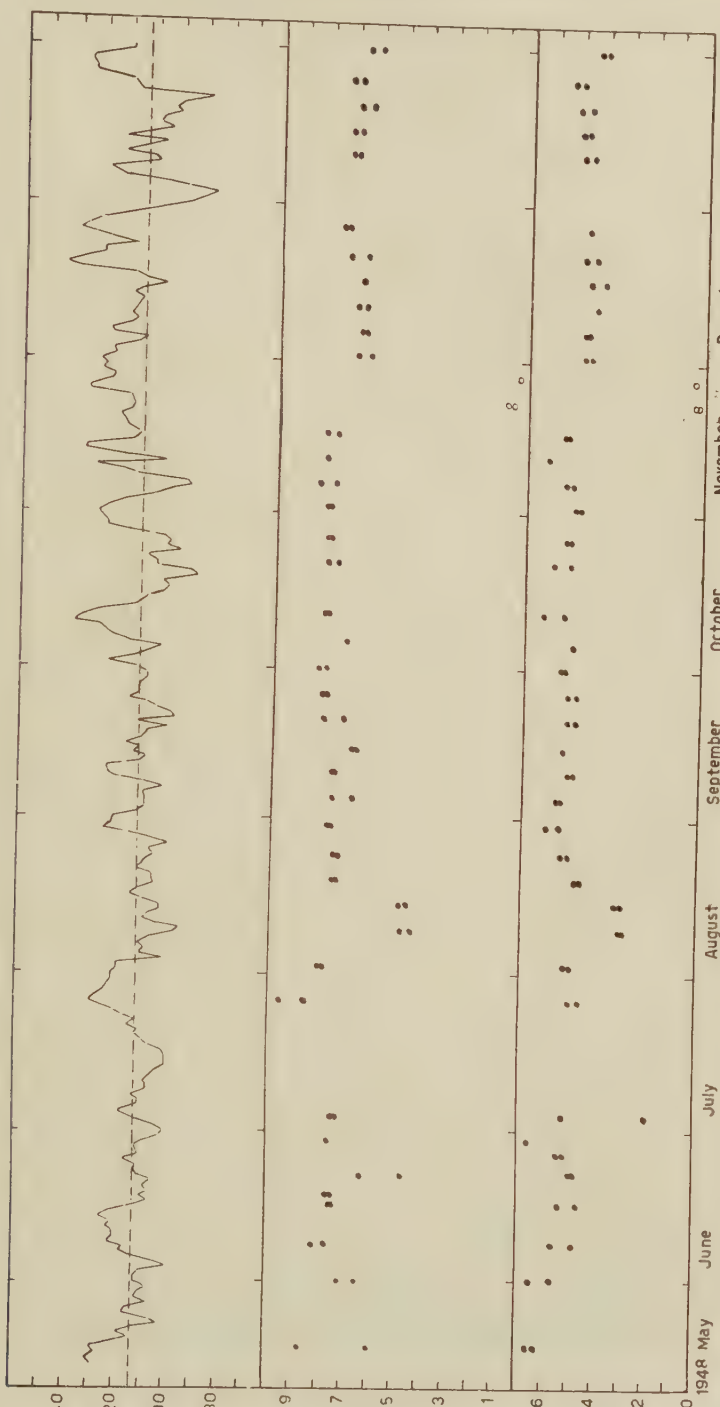

Fig. 1. Nitrogen fixation and daily changes in atmospheric pressure, May 1948–January 1949. (Black dots indicate duplicate cultures; in case of very closely agreeing duplicates only one dot is shown).

CuO. Digestion was continued for 3 hours after clearing of the mixture; N/28 H_2SO_4 and NaOH were used for the titration which took place in CO_2-free solution with methyl red + methylene blue as indicator. Blank corrections for nitrogen in medium + inoculum + reagents were obtained by analyzing cultures immediately after inoculation. Analyses of 40 blanks distributed over the whole experimental period showed a nitrogen content varying from 0.10 to 0.28 mg, with a mean of 0.16 and a standard deviation of $\pm$ 0.046 mg.

Altogether 83 experiments were performed with each strain, in all except two cases simultaneously. As a rule the termination of each experiment coincided with the beginning of the next. A few experiments were lost through technical mishaps, and on some occasions (e.g., July 1948) external circumstances made a longer interval unavoidable. One of the duplicates in two experiments with each strain were lost accidentally; the total set of data thus comprises 164 single nitrogen determinations on each strain.

The general course of the nitrogen fixation is seen in Figg. 1 and 2 which also show the variations in air pressure (daily readings at midnight, taken from the daily weather bulletins of the Meteorological Institute of Copenhagen, situated about 12 km from the laboratory; 1000 millibar = 750 mm at 0°C). The results from the first four months of 1948 seem to agree with those of Bortels, at least as far as strain *A* is concerned. A decline in nitrogen fixation about the middle of June coincides with a low-pressure period, and in late July a maximum is reached during a period of fine summer weather with prevailing high barometric pressure. The gains by strain *D* during the period, however, only show minor fluctuations (apart from an abnormally low value in one duplicate of a single experiment in early July), and these do not coincide with weather changes; a peak in late May actually occurs during a period of low pressure, long before a high-pressure area approaches. During the first half of August, two consecutive experiments with both strains show a very marked drop in nitrogen fixation, coinciding with the onset of a period of low air-pressure, rains and thunderstorms. However, the subsequent recuperation is not accompanied by the immediate approach of a high pressure, and from now until the middle of November the gains remain at a high and fairly constant level, in spite of very drastic atmospheric changes in October and early November. An extraordinary phenomenon appears about the middle

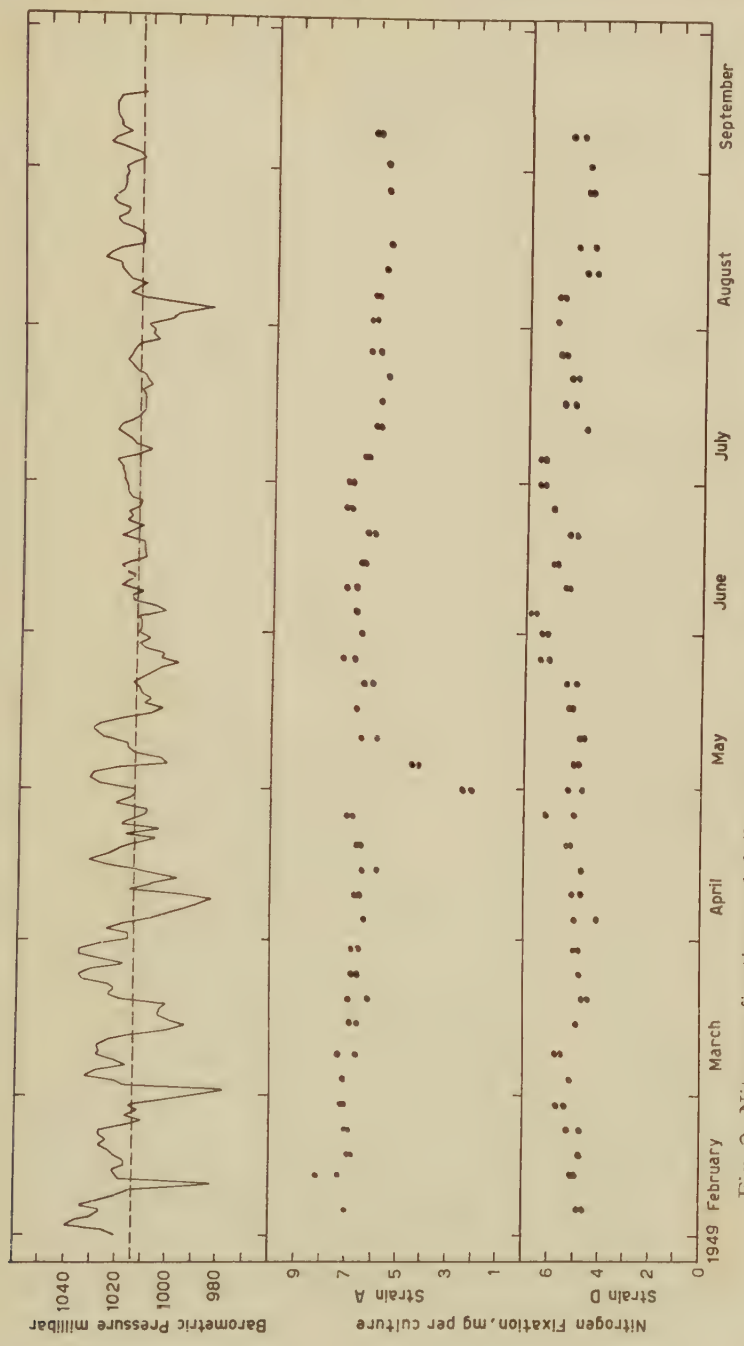

Fig. 2. Nitrogen fixation and daily changes in atmospheric pressure, February–September 1949.

of November when both strains in two consecutive experiments almost fail to fix nitrogen (and to consume glucose). No marked atmospheric changes take place, the air-pressure being mostly fairly high, and it is difficult to avoid the suspicion of some defect in the medium of which the same batch was used for both experiments. The recuperation from the depression coincides approximately with a drop in atmospheric pressure. From now on until late April 1949 the gains appear fairly steady, and no response is shown by either strain to a low pressure in late December or a very marked high-pressure in late January and early February. It is noteworthy that the gains in both strains after the November depression are on a lower level than before it, and remain so in strain *A* which shows an exceptional peak in early February at a time when Copenhagen was at the centre of a very marked although transient low-pressure area! In strain *D* the figures reach the pre-depression level during February, with some fluctuations that show no obvious correlation with the atmospheric changes. In late April and early May strain *A* shows another depression which does not affect strain *D* and thus obviously is not due to the medium, and which is not accompanied by any conspicuous atmospheric change. The growth of strain *A* after this depression shows no great fluctuations, but a slow and rather steadly decline towards the end of the experimental period. Strain *D*, on the other hand, shows its very highest gains in late May and early June during a prolonged low-pressure period, and another interval of high activity in early July when no great atmospheric changes are observed. Finally the last five experiments show again somewhat lower figures, and this during a period when the atmospheric pressure is mostly above the normal.

A general correlation between growth and atmospheric changes is thus not discernible. The marked depressions have only in one instance coincided with the onset of a low-pressure period, whereas several drastic changes between high and low pressure have failed to influence the growth. To see if the observed variations in nitrogen fixation can be explained as due to chance variation, the figures (omitting the results of the two obviously abnormal experiments in November 1948) were divided into groups of 0.5 mg nitrogen and the frequencies in the various groups compared with those expected if the figures were normally distributed. The result is seen in Table 1.

TABLE I

Distribution of gains of nitrogen by *Azotobacter* in 160 analyses

Nitrogen fixed, mg	Strain A		Strain D	
	Frequencies		Frequencies	
	Observed	Expected	Observed	Expected
1.50	0	0	1	0
2.00	2	0	0	0
2.50	0	0	1	0
3.00	0	1	3	1
3.50	0	1	0	6
4.00	2	2	10	20
4.50	5	4	45	38
5.00	0	10	60	45
5.50	18	18	22	32
6.00	23	27	13	14
6.50	42	31	5	4
7.00	32	28	0	0
7.50	23	20		
8.00	10	11		
8.50	2	5		
9.00	0	2		
9.50	1	0		
10.00				
Total.	160	160	160	160
Nitrogen fixation, mg.				
Mean.	4.79		5.16	
Standard deviation.	1.008		0.692	

In both strains the distribution of the figures is approximately symmetric, but with a quite marked tendency to excess around the mean, especially in strain *D*. A few excessive values on the negative side also occur; this are the figures corresponding to the clear-cut depressions and the single abnormal duplicate in strain *D* (9 July 1948).

A closer inspection of the graphs in figs. 1 and 2 reveal certain long-term variations, particularly in strain *A* where the gradual decline in nitrogen fixation is obvious. This is brought out more clearly if we calculate the average nitrogen fixation in the various periods outside the three depression periods. This is shown in Table II.

Strain *D* shows a significantly lower fixation only in the third period (the high standard error in the first period is largely due to a single abnormal culture), but in strain *A* there is a further significant drop in the last period. The cultures of strain *A* that had been continually

TABLE II

Periodical gains of nitrogen by *Azotobacter*				
Period	Number of analyses		Nitrogen fixed, mg (Mean and standard error)	
	Strain *A*	Strain *D*	Strain *A*	Strain *D*
1. From start to beginning of the first depression (18/5–2/8-1948)	19	19	7.44 ± 0.249	5.16 ± 0.218
2. From end of first to beginning of second depression (19/8–16/11-1948)	34	33	7.66 ± 0.069	5.43 ± 0.066
3. From end of second to beginning of third depression (1/12–('48)–25/4 ('49)	54	54	6.84 ± 0.051	4.89 ± 0.054
4. After third depression to end of experiment (10/5–7/9–1949)	45	46	6.29 ± 0.075	5.48 ± 0.099

transferred on agar medium were found to have suffered some loss of nitrogen-fixing power. A culture of strain *A* in sterilized soil had been made in September 1948, when the strain was at the height of its nitrogen fixation. When the last five experiments were performed in August–September 1949, extra duplicate flasks were inoculated from a primary agar culture from this soil culture. The results (Table III) show clearly the superiority of the culture that had been maintained in soil.

TABLE III

Nitrogen fixation by strain *A*. Comparison between permanently agar-transferred culture and primary agar culture from soil				
Date of experiment	Gain of nitrogen, mg per culture, with inoculum			
	Permanently agar-transferred		Primary agar-culture from soil culture	
	a	*b*	*a*	*b*
6/8–11/8–1949	5.69	5.74	7.01	7.21
11/8–16/8–1949	5.59	5.63	6.92	7.35
22/8–27/8–1949	5.64	5.65	6.50	6.89
27/8–1/9–1949	5.64	(lost)	6.78	6.79
2/9–7/9–1949	5.97	6.08	6.96	7.44

Plating from the agar-transferred cultures after the experiment revealed the presence of two colony-types resembling a "rough" and a "smooth" variant. These were compared with inoculum from the soil culture (primary agar culture) in an experiment where glucose

consumption as well as nitrogen fixation was estimated. Like the previous, these cultures were incubated for 5 days at 25°C. The initial glucose supply was 480 mg per flask, which in all cases had been used up at the end of the experiment. The results are shown in Table IV.

TABLE IV

Nitrogen fixation by *R*- and *S*-type of Strain *A* compared with primary agar culture from soil

Nitrogen fixed, mg:		R-Type	S-Type	Soil Culture
Per culture:	*a*)	5.91	6.54	7.64
	b)	5.99	5.71	7.66
	c)	5.65	5.91	—
Per g glucose consumed:	*a*)	12.3	13.6	15.9
	b)	12.5	11.9	16.0
	c)	11.6	12.3	—

The *R*-type and the *S*-type show no significant difference, but their nitrogen-fixing efficiency (gain per g of consumed glucose) is definitely lower than that of the culture maintained in soil, which in this experiment has fixed as much nitrogen as at the time when the soil culture was made (September 1948). The fact that the glucose was all used up suggests that the change in the agar-transferred cultures did not involve a reduced growth rate, but an actual loss of nitrogen-fixing power (lessened production of nitrogen-fixing enzyme?). It appears from the graphs in fig. 1 that the variant arose in December 1948, and that with progressing time it gradually became more and more predominant in the cultures.

The fact that the nitrogen fixation figures in Table I are approximately normally distributed, with excess around the mean, suggests that the smaller variations may be largely due to minor accidental and unavoidable differences in the composition and preparation of the medium *), the inocula, temperature of incubation, etc., whereas

*) For instance, small differences in temperature during sterilization may cause some variation in nitrogen fixation, as shown by an experiment with strain *A* (from soil culture, 16/1–21/1-1950):

Mg N fixed per culture in medium sterilized by:

	Filtration:	Autoclaving 20 min. at:			
	—	110°C	115°C	120°C	125°C
a)	7.86	8.08	7.52	7.06	6.75
b)	8.18	8.13	7.72	—	6.88

This is obviously not the cause of the variations in Bortels' experiments, where the medium was not sterilized at all.

the more pronounced depressions and the long-term variations must be ascribed to other causes. But since the long-term variations do not run parallel in the two strains, they can hardly be due to a common external factor, such as the radiation assumed by Bortels. In strain *A* this variation is evidently connected with the arising of a mutant with lower nitrogen-fixing power. The possibility that a physical factor like cosmic radiation could induce mutation-like changes in the bacteria certainly cannot be dismissed, but the present data give no evidence that the operation of such a factor is correlated with atmospheric changes, or that these latter have any connection with the abnormal depressions. A direct explanation for the disagreement with the results of Bortels is not yet possible, but at least it seems certain that the meteorological phenomena do not affect all strains of *Azotobacter*.

Summary

Periodical nitrogen fixation experiments with two strains of *Azotobacter chroococcum* for 16 months showed no definite correlation between gains of nitrogen and meteorological changes, as reported by Bortels. Variability in the gains of nitrogen seemed to be due largely to chance variation, together with certain abnormal depressions which did not clearly coincide with changes in atmospheric pressure. The conclusions of Bortels thus do not seem to be generally valid for *Azotobacter*. Certain long-term variations in nitrogen fixation seemed in one strain to be connected with the arising of a variant of lower nitrogen-fixing power.

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REFERENCES

1) Allison, F. E., Gaddy, V. L., Pinck, L. A., and Armiger, W. H. Azotobacter Inoculation of Crops. II. Soil Sci., **64**, 489–497 (1947).
2) Bortels, H., Meteorobiologische Beobachtungen an *Azotobakter*. Zentr. Bakt. Parasitenk., II, **102**, 129–153 (1940).
3) Bortels, H., Über Beziehungen zwischen epidemiologischem und meteorologischem Geschehen (etc.). Zentr. Bakt. Parasitenk., II, **104**, 289–325 (1942).
4) Bortels, H., Meteorobiologische Reaktionen einiger Mikroorganismen. Zentr. Bakt. Parasitenk., II, **105**, 305–325 (1942).
5) Bortels, H., Über die Abhängigkeit der Virulenz und anderer Eigenschaften pathogener Bakterien sowie des Infektionserfolges vom Wetterverlauf. „Festschrift Appel", Berlin-Dahlem (1947).
6) Fåhraeus, G., Nilsson, R., Sundelin, G., and Rasmussen, J. Behandling av utsäde med azotobacterkultur, radivit och euradin. Kungl. Lantbrukshögskolan (Uppsala), Medd. No. 24 (1948).
7) Hansen, Sigrid, and Schwartz, W., Untersuchungen über die Einwirkung von ultrakurzen Wellen auf Mikroorganismen. Arch. Mikrobiol., **13**, 219–249 (1943).
8) Stapp, C., and Bortels, H., Azotobakterwachstum und Stickstoffbindung in Abhängigkeit vom Wetter. Zentr. Bakt. Parasitenk., II, **94**, 497–499 (1936).

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FIXATION DE L'ACIDE PHOSPHORIQUE SUR LE CALCAIRE DES SOLS

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L'étude du comportement de l'acide phosphorique soluble apporté aux sols calcaires a fait l'objet de nombreuses recherches. Ces travaux ont été effectués le plus souvent avec des solutions relativement concentrées.

Le but de ce travail, au contraire, est de préciser comment se comportent les solutions du sol a faible concentration (de l'ordre de 1 à 3 mg P_2O_5 par litre) lorsqu'elles arrivent en contact avec les grains de sable calcaire.

Seuil de precipitation. 25 cm^3 d'une solution de phosphate monocalcique à 1 mg P_2O_5 p. litre contenant une quantité de $CaCl_2$ correspondant à 0.03 N étaient additionnés de 25 cm^3 d'eau renfermant une quantité d'eau de chaux croissante pour élever progressivement le pH.

En opérant de cette facon, nous constatons que le P_2O_5 ne commencait à précipiter qu'à un pH 8.5/9.

Lorsque nous dépassons pH 9, les phosphates précipitent mais repassent en solution sous l'action du CO_2 de l'air qui acidifie le milieu.

Ces constations préliminaires jointes a celles de B a r b i e r, nous montrent qu'avec des solutions contenant 0 à 3 mg P_2O_5 par litre nous n'acons pas à craindre de précipitation au contact des grains calcaire dont le pH est voisin de 8.5.

Fixation de P_2O_5 sur les grains calcaires. T e c h n i q u e d e s e x p é r i e n c e s. Pour suivre la fixation de P_2O_5 aur le clacaire, on pèse une quantité voulue de CO_3Ca, on le verse dans un bécher avec 50 cm^3 d'une solution de phosphate monocalcique. On agite avec une baguette de verre et on filtre aprés des temps variables; on centri-

fuge et on dose P_2O_5 restant dans le liquide par la méthode coeruléo-molybdique.

Influence du temps de contact. Nous avons ajouté 50 cm^3 d'une solution de phosphate (à 2,2 mg de P_2O_5 par litre) avec 0,5–1 et 2 g de CO_3Ca par précipité. Aprés 30 min., 15 h et 46 h de contact, le P_2O_5 non fixé a été dosé dans le liquide.

Les quantités de P_2O_5 fixé, calculées par différence, ont été les suivantes:

TABLE I

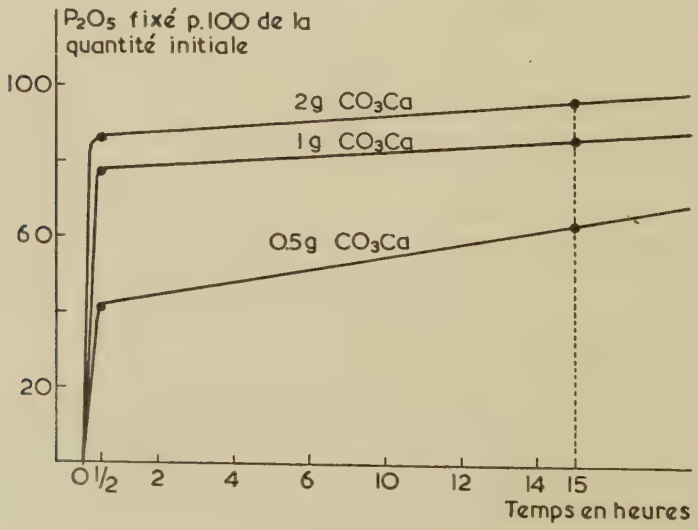
Co_3Ca mis en expérience	P_2O_5 fixé per cent de la qualité introduite aprés:		
	30 min.	15 h.	46 h.
0,5 g	41	63,5	71
1 g	77	86,5	87
2 g	86,5	95,5	96

Il résulte de ces chiffres:

1°. Une forte proportion d'acide phosphorique est fixée pendant les 30 premières min, puis le phénomène se prolonge ensuite très lentement.

2°. La quantité de P_2O_5 fixée n'atteint jamais 100 p. cent de la quantité introduite.

Le calcaire ne fixe donc pas instantanément et en entier le P_2O_5 comme cela aurait lieu s'il s'agissait d'une précipitation chimique.

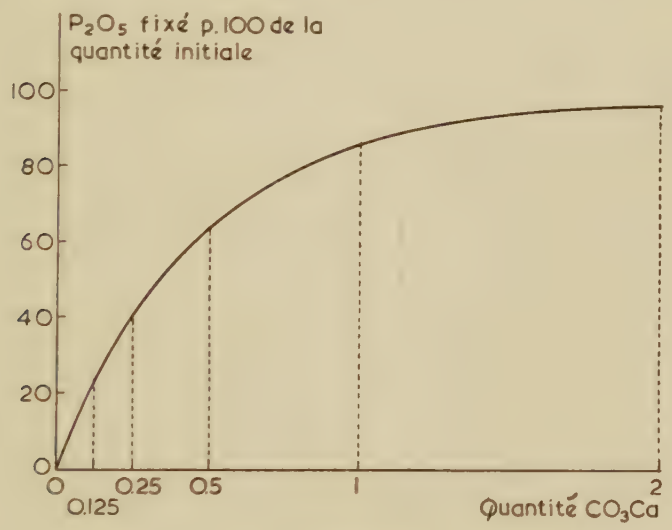


Graphique 1.

Influence de la quantité de CO_3Ca. A la même solution de phosphate (2,2 mg P_2O_5 par litre) on ajoute des quantités de CO_3Ca variant de 0,125 à 2 g. Les quantités de P_2O_5 fixé p. cnt des quantités introduites, sont les suivantes:

Pour	2	g CO_3Ca	96,5
,,	1	g ,, ...	86,5
,,	0,5	g ,, ...	63,5
,,	0,25	g ,, ...	41
,,	0,125	g ,, ...	23

Le graphique ci-contre montre la marche de la fixation en fonction de la quantité de calcaire (graph. 2).



Graphique 2.

Si nous considérons maintenant non la quantité de P_2O_5 fixé sur la totalité de CO_3Ca, mais le quantité fixée par gramme de calcaire mis en expérience, nous trouvons:

TABLE II

Quantité de CO_3Ca mis en expérience	P_2O_5 fixé par g de CO_3Ca (en γ)
2 g	52
1 g	99
0,5	154
0,25	176
0,125	190

A un même poids de CO_3Ca ne se fixe donc pas le même quantité de P_2O_5 comme cela aurait lieu s'il s'agissait d'une combinaison chimique.

Influence de la finesse des grains. A poids égal, la quantité fixée est fonction de la finesse des grains calcaires; ainsi pour 1 g de marbre blanc pulvérisé, une solution à 1 mg P_2O_5 par litre est fixée de la façon suivante:

Diamètre des grains de 100 à 160 μ. . . . . 50 γ
„ „ „ „ <75 μ . . . . . 114 γ

Influence de la concentration en P_2O_5. Le tableau suivant résume les résultats obtenus.

TABLE III

Concentration initiale en P_2O_5 mg par litre	Poids de CO_3Ca mis en expérience					
	125 mg		250 mg		500 mg	
	Concentration finale P_2O_5	P_2O_5 fixé en γ par g de CO_3Ca	Concentration finale P_2O_5	P_2O_5 fixé en γ par g CO_3Ca	Concentration finale P_2O_5	P_2O_5 fixé en γ par g de CO_3Ca
20,4	—	—	0,05	38	0,03	21
0,48	0,24	96	0,13	70	0,07	41
0,96	0,60	144	0,45	102	0,21	75
1,95	1,50	180	1,20	150	0,84	111
3,0	2,50	200	2,14	172	1,44	156
5,0	4,20	240	3,80	240	3,00	200
10,0	8,75	340	8,50	300	7,00	300

Si l'on porte en ordonnée la quantité fixée et en abscisse la quantité restant en solution, nous obtenons les 3 courbes suivantes.

En coordonnées logarithmiques, le début de chacune de ces trois courbes est représenté sensiblement par une droite.

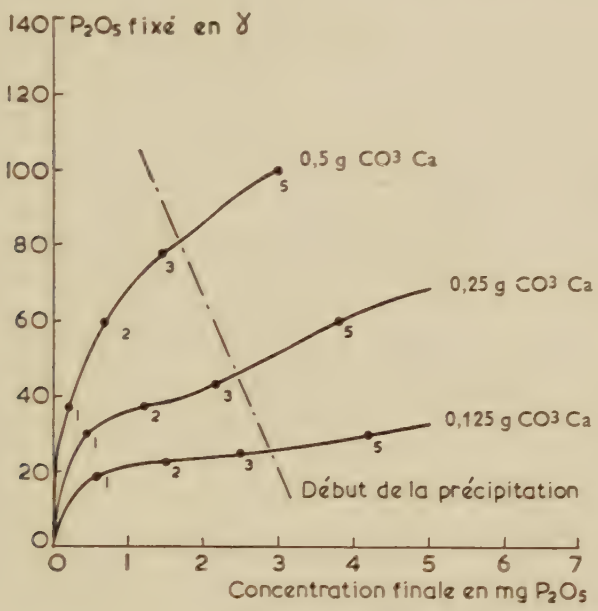
Nous voyons donc que jusqu'à une concentration initiale de 3,5 mg par litre, nous obtenons une courbe indiquant qu'il s'agit d'une adsorption.

Au-dessus de cette concentration, la fixation ne suit plus les lois de l'adsorption, mais augmente d'une facon linéaire. Il y a donc précipitation d'une phosphate complexe gélatineux comme il a été indiqué d'autre part.

Le seuil de précipitation, bien indiqué par ce graphique se situe donc vers 3,5 mg P_2O_5 par litre pour un pH de 8,3 à 8,5. Ce seuil de

*) P. Boischot, Ann. Agr. 1934, p. 77.

précipitation est le même que l'on opère avec des grains calcaires ou avec une solution d'eau de chaux.



Graphique 3.

Pendant la première demi-heure de contact, temps nécessaire à la fixation, le pH mesuré à l'électrode de verre subit les variations suivantes:

TABLE IV

	Poids de CO_3Ca mis en expérience					
	125 mg		250 mg		500 mg	
	Solution à 0,25 mg P_2O_5 par litre	Solution à 10 mg P_2O_5 par litre	Solutoin à 0,25 mg P_2O_5 par litre	Solution à 10 mg P_2O_5 par litre	Solution à 0,25 mg P_2O_5 par lire	Solution à 10 mg P_2O_5 par litre
p_H au moment de la mise en suspension du CO_3Ca dans la solution	9.2	8.4	9.3	8.3	9.4	8.4
Suspension de CO_3Ca après 20 min d'agitation .	8.4	7.9	8.5	8.0	8.7	8.1
p_H du liquide après centrifutation et séparation de CO_3Ca	8.2	7.7	8.3	7.8	8.5	7.9

On voit donc, qu'au moment de la mise en suspension du CO_3Ca dans la solution de phosphate, la pH peut d'élever au-dessus de 9: Un début de précipitation est donc possible, mais le pH redescendant rapidement par suite de l'action du CO_2 atmosphérique, les phosphates précipités repasseraient en solution, comme nous l'avons vu précédemment.

Fixation en milieu acide. Nous avons étudié le phénomène en milieu acide dans la mesure ou la présence du calcaire est compatible avec cette acidité.

Pour cela la suspension de CO_3Ca dans la solution de phosphate est additionnée de l'acide sulfurique nécessaire pour amener le pH a 4,5 et 5,5.

Par suite de la dissolution d'une partie de CO_3Ca, le pH remonte d'une unité environ après 20 min, temps au bout duquel P_2O_5 resté dissous est dosé; nous avons obtenu les chiffres suivants:

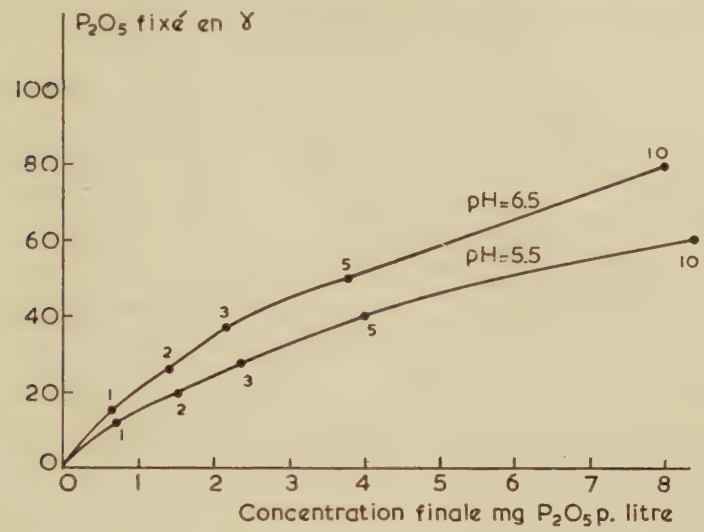
TABLE V

concentration initiale en P_2O_5 mg her litre	p_H final 6,5		p_H final 5,5	
	concentration finale en P_2O_5 mg par litre	P_2O_5 fixé en γ par g de CO_3Ca	concentration finale en P_2O_5 mg par litre	P_2O_5 fixé en γ par g de CO_3Ca
0.48	0.34	28	0.36	24
0.96	0.66	60	0.71	50
1.92	1.40	104	1.52	80
2.88	2.15	146	2.35	112
4.80	3.80	200	4	160
9.60	8	320	8.4	240
24.00	19.5	900	—	—

En tracant les courbes comme précédemment, nous observons un phénomène d'allure logarithmique et ceci jusqu'à de fortes concentrations en P_2O_5, puisqu'aucune précipitation ne peut se produire en milieu acide.

La convexité des courbes est toutefois peu accusée, ce qui dénote une faible énergie de rétention des petites doses de phosphates.

Mobilisation de P_2O_5 ainsi fixé. 2 g de calcaire ayan adsorbé 106 γ de P_2O_5 ont été agités pendant $^1/_4$ d'heure avec une solution d'humate d'ammonium neutre à 1 p. 1000 se rapprochant de la solution du sol.



Graphique 4.

Si nous faisons varier la quantité de solvant, nous trouvons:

TABLE VI

Solution d'humate d'ammonium p_H 7 cm³	Reste fixé en γ	P_2O_5 extrait en	concentration (γ P_2O_5 dans 1000 cm³)
50	102.5	3.5	70
100	100.7	5.3	53
200	97.6	8.4	42
500	92.3	13.7	27.4
1.000	84	22	22

La quantité de P_2O_5 extrait p. 1000 de solution diminue lorsque le volume de solution augmente ce qui correspond bien a un phénomène de désorption:

En résumé:

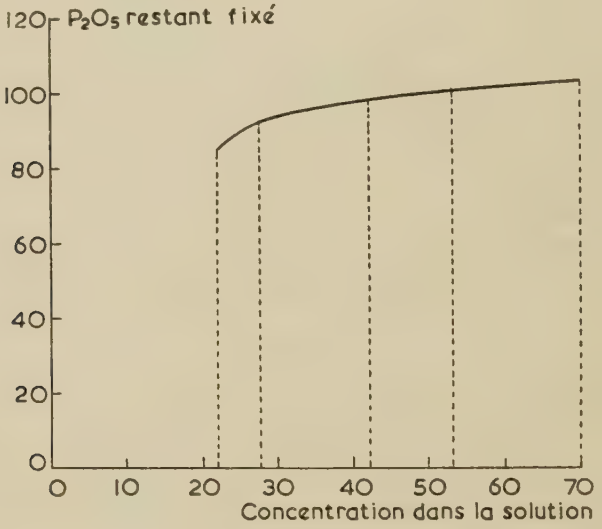
1) La fixation de P_2O_5 à faible concentration sur les grains calcaires et dû à un phénomène d'adsorption,

2) Le P_2O_5 ainsi fixé peut être remis en solution par un phénomène de désorption par les liquides du sol.

Etude sur le calcaire des sols. Si le P_2O_5 des solutions du sol se fixe de cette façon sur les grains calcaires de la terre

arable, nous ne devons pas avoir dans les sols de précipité de phosphate, mais bien une mince couche de P_2O_5 adsorbé autour des grains de CO_3Ca.

Pour nous en rendre compte, nous avons séparé les parties sableuses d'une terre de Grasse (Alpes Maritimes) en fractions de différentes grosseurs.



Graphique 5.

Dans chaque fraction dont l'ensemble représente les sables grossiers et fins, nous avons dosé CO_3Ca et P_2O_5 solubles dans les acides concentrés.

TABLE VII

Refus tamis No.	Diamétre des particules en μ	Calcaire p. cent	P_2O_5 total p. mille
18	1.000 à 2.000	90	0.35
30	500 à 1.000	75	0.50
60	200 à 500	65	0.80
90	160 à 200	57	1.20
150	100 à 160	51.5	1.60
200	75 à 100	46.5	2.40

En calculant les quantités d'acide phosphorique fixées par rapport à la surface et au poids des grains sableux, nous obtenons:

TABLE VIII

Diamétre moyen des grians en μ	Surface de 1 g de sable en cm²	Poids en mg CO_3Ca par g de sable total	Surface en cm² des grains calcaires p. 1 g de sable	P_2O_5 en γ par g de sable	P_2O_5 en γ par cm² de calcaire
(1)	(2)	(3)	(4)	(5)	(6)
1.500	15	900	13.5	350	25.9
750	30	750	22.5	500	22.2
350	75	650	48.7	800	16.5
180	122	570	69.3	1200	17.2
130	172	515	87.3	1600	18.3
87.5	258	465	120.0	2400	20.0

1) Le P_2O_5 est fixé à la surface des grains calcaires. Dans ce cas, la quantité d'acide phosphorique dans les différentes fractions doit être proportionnelle non pas au poids du CO_3Ca qu'elles renferment mais bien à la surface des grains calcaires qui varie en raison inverse de leur diamètre.

Nous ne pourrons d'ailleurs avoir qu'une approximation car nous avons calculé les surfaces en prenant le diamètre moyen des grains et en supposant que toutes les particules sont égales et sphériques et possédent une densité uniforme de 2,7. Ces hypothéses nécessaires pour le calcul ne sont évidemment pas rigoureusement exactes dans la réalité.

Le tableau ci-dessus résume les principaux résultats que nous avons obtenus:

Il nous montre qu'alors que la quantité de calcaire varie de 1 à 0,5 (colonne 3), la quantité d'acide phosphorique varie de 1 à 7 (col. 5) dans le mëme ordre de grandeur que celle de la surface des grains calcaires: 1 à 8 (col. 4); la surface de l'ensemble des sables calcaires et siliceux varie de 1 à 17 (col. 2).

Si nous faisons abstraction de la première fraction dont les résultats peuvent ëtre faussés du fait de la grosseur des grains (non sphéricité-possibilité de fissures augmentant la surface, etc.), nous voyons que la concordance entre la surface du calcaire et la teneur en P_2O_5 est encore plus frappante puisque cette surface varie de 1 à 5,3 et le P_2O_5 de 1 à 4,8.

En conséquence:

1°. La quantité du P_2O_5 est sensiblement proportionnelle à la surface des grains calcaires (col. 4 et 5) autrement dit la quantité d'acide

phosphorique est sensiblement constante par unité de surface de ces grains (col. 6).

2°. Elle ne dépend ni de la surface du sable total (col. 2) ni du poids du calcaire (col. 3).

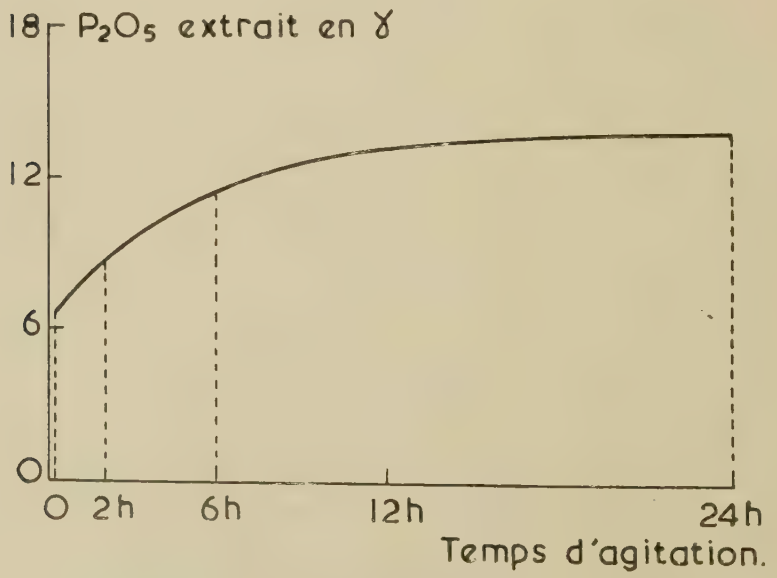
3°. La quantité de P_2O_5 fixé sur le sable quartzeux est insignifiante comme l'a montré expérimentalement A. T. Perkins.

2) L'extraction du P_2O_5 est bien un phénomène de désorption. Influence du temps d'extraction: Nous avons agité 500 mg du sable calcaire extrait de la terre précédente, et d'une grosseur de 500 à 1000 avec 500 cm^3 d'eau pendant des temps variables. Nous avons extrait les quantités de P_2O_5 suivantes:

TABLE IX

Temps d'agitation	P_2O_5 extrait en γ
1/4 d'heure	6,8
2 h	7.8
6 h	11,4
24 h	13,6

Nous voyons en tracent la courbe que l'on a bien les caractéristiques d'un phénomène de désorption (graph. 6).



Graphique 6.

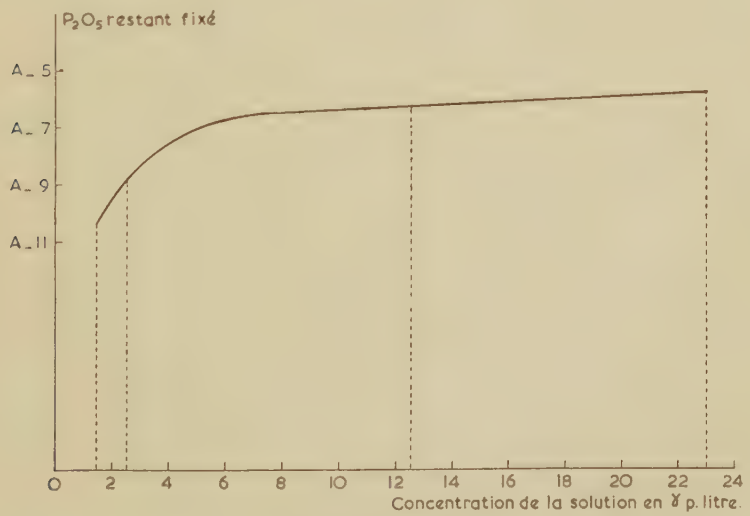
Influence du rapport CO_3Ca/liquide: Si nous maintenons fixe la quantité de sable calcaire (500 mg) et que nous faisons varier le volume d'eau servant à l'extraction, les quantités de P_2O_5 extraites sont, après 2 h d'agitation:

TABLE X

Volume du liquide en cm³	P_2O_5 extrait en γ	Concentration en P_2O_5 (γ par litre)	Quantité de P_2O_5 restée fixée
25	5,8	23,2	A — 5,8
50	6,4	12,8	A — 6,4
250	7,5	3,0	A — 7,5
350	8,9	2,5	A — 8,9
750	11,5	1,5	A — 11,5

Le tableau ci-dessus et la courbe VII obtenue en mettant en abcisse la concentration de la solution et en ordonnée la quantité de P_2O_5 demeurée fixé (à une constante près) nous montrent que le P_2O_5 extrait p. mille augmente moins que proportionnement au volume du liquide d'extraction, ce qui est bien le propre d'un phénomène de désorption.

Par exemple quand le volume de la solution passe de 1 à 30 la quantité de P_2O_5 extraite nefait que doubler (graph. 7).



Graphique 7.

Conclusion. Les expériences ci-dessus nous montrent que le P_2O_5 des solutions du sol à faible concentration se fixent sur les grains calcaire de la terre arable non par précipitation, mais par un phénomène d'adsorption.

L'acide phosphorique ainsi fixé peut repasser en solution par le phénomène inverse de désorption pour être mis a la disposition des plantes lorsque le titre de la solution du sol en P_2O_5 s'appauvrit.

Lorsqu'on ajoute au sol des engrais phosphates, la concentration en P_2O_5 autour des grains d'engrais peut ëtre suffisamment élevée pour qu'il y ait précipitation de phosphate gélatineux autour des granules d'engrais.

Mais ces précipités se redissolvent au moins partiellement dans les solutions du sol et se fixent sur les grains calcaire suivant le processus que nous venons d'indiquer.

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MANGANESE IN RELATION TO PHOTOSYNTHESIS

III. UPTAKE OF OXYGEN BY ILLUMINATED CRUDE CHLOROPLAST SUSPENSIONS

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Introduction. Since the redoxpotential of a crude suspension of *Avena* chloroplasts quickly increases by a hundred millivolts or more within a few minutes of the commencement of illumination [2]) in the presence of oxygen, the question may be raised as to whether illumination has any influence on the uptake of oxygen by such a suspension. Hill [4]) showed that oxygen was not liberated by chloroplasts suspended in water unless leaf extract was added. Therefore it might be expected that in the case of crude suspensions of chloroplasts, in which the chloroplasts are mingled with cell contents, oxygen will similarly be liberated under these conditions.

On the other hand the high potentials, of 500 mV, or more, observed in illuminated crude suspensions of chloroplasts, to which manganese had been added, undoubtedly favour the oxidation of cell constituents, a number of which are easily dehydrogenated. Under these circumstances oxygen will be used and ultimately carbon dioxide will be produced which will be reflected by changes in the pH of the suspension.

It would, therefore, be of interest to ascertain the effect of addition of specific inhibitors of respiration, such as HCN, on the uptake of oxygen by such a system and further, the effect of addition of individual cell constituents such as glucose and amino acids.

Due to circumstances already mentioned in the first paper these investigations could only be carried out on a limited scale.

Experimental methods. Uptake of oxygen was measured by means of Warburg-Barcroft respirometer, the flasks used were of

the flat round type having a diameter of 55 mm, a height of 10 mm and a small central well containing a fan shaped piece of filter paper soaked in 40% KOH solution to absorb any carbon dioxide liberated. To each flask, whose total capacity was 30 ml, was added 8 ml of chloroplast suspension prepared in the usual way [2]). The flasks were gently shaken during the experiments to ensure a rapid exchange of gases. The constant for the apparatus was determined by the slightly modified method of Münzer and Neumann [7]) as described by Umbreit [14]) (p. 56). This is carried out by removing known quantities of gas and noting the corresponding thermo-barometer readings. In the present experiments the calibrated pipette was provided with a levelling bulb containing water instead of mercury which ensured greater accuracy.

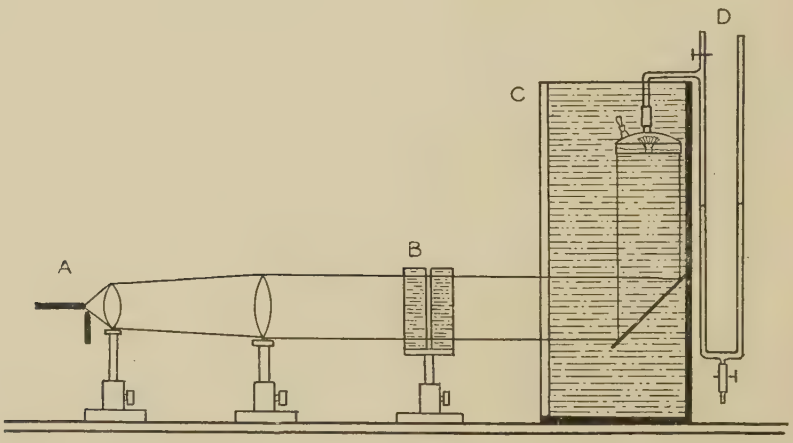


Fig. 1. Apparatus used in investigation of the effect of illumination on the uptake of oxygen by crude suspensions of *Avena* chloroplasts.

a. carbon arc lamp.
b. filters for absorption of heat rays.
c. Constant temperature water bath with mirror.
d. Warburg-Barcroft thermo-barometer.

The flasks, submerged in a thermostatically controlled waterbath, were illuminated from below by a beam of light from a carbon arc lamp (Zeiss 4. A.d.c.). The beam of light, concentrated by two convex lenses, was filtered through glass cells containing water and a

solution ,of $FeSO_4$ respectively *) and then reflected by a mirror placed below the flasks and in the waterbath as shown in fig. 1. Although most of the heat rays were absorbed before the light reached the surface of the experimental flask a slight increase of pressure inside the respiration flask occurred when the light was switched on and could not be avoided; similarly a decrease of pressure took place when the light was switched off.

Since the arc lamp was automatically regulated the light intensity was constant and the resulting increases and decreases of pressure were constant and of the same magnitude; this made it easy to correct the manometer readings.

The plant material was the same as that used in the previous experiments and consisted of the filtrate from crushed *Avena* leaves centrifuged at low speed and suspended in water or in a diluted buffer solution containing 0.01—0.005 mol. KH_2PO_4 and K_2HPO_4 (pH 6.5) or $NaHCO_3$ and 0.1% KCl, 0.1% $MgSO_4$ and 0.025% $CaSO_4$.

In those cases where various determinations had to be made on the same plantmaterial the leaves were divided in half lengthwise and one half wrapped in moist filter paper and stored in the refrigerator at 2°C for a few hours.

Results

Uptake of oxygen by crude suspensions of chloroplasts. Experimental Material used. Experimental material. Second leaf from top of healthy oatplant, grown in the greenhouse under conditions of artificial illumination for three months between September and December. 1,5 g in 25 ml buffersolution; 8 ml in Warburg flask. Air above the suspension.

From the results obtained, which are summarised in the following table, it is evident that the suspension takes up oxygen in the dark, the rate of uptake gradually diminishing until, after $1\frac{1}{4}$ hours in the dark, the respiration rate is only 25% of the original respiration rate.

At the commencement of the illumination period the uptake of oxygen showed a sixfold increase, compared with the quantity of oxygen taken up during the last 10 minutes of the preceding dark

*) The solution contained 15% $FeSO_4$, 1% glucose, 2% H_2SO_4 covered with a thin layer of paraffin oil. The reducing action of the glucose prevents the oxidation of the ferrosulfate. A 3 cm layer absorbs the greater part of the heat and infra red rays, and only 10 to 15% of the visible light.

TABLE I

Uptake of Oxygen by a crude suspension of chloroplasts				
	Time in Minutes	Total mm^3 O_2 taken up from beginning of experiment	mm^3 O_2 per min. taken up in successive periods	mean values for mm^3 O_2/min. taken up during the periods of the darkness and illumin.
Dark	12	14	1.2	0.88
	36	42	1.2	
	45	51	1.0	
	65	66	0.8	
	75	69	0.3	
Light	78	—	—	2.10
	81	71		
	90	88	1.8	
	100	114	2.6	
	110	135	2.1	
	120	153	1.8	
	130	175	2.2	
Dark	131	—	—	0.14
	136	184	0.2	
	141	185	0.1	
	151	186	0.08	
	164	187		

period, and a maximum rate of 2.6 mm^3 min. was reached after 12 minutes illumination. The mean rate of uptake of oxygen during the subsequent dark period was only 6.2% of that during the preceding illumination period. During the second dark period the decrease in rate of uptake of oxygen, already observed during the first dark period, continued. It seems that the material which is capable of being oxidised in the dark becomes exhausted after one hour whereas the substance which are oxidised during illumination are present in greater quantities.

The striking difference between the results obtained in the present experiments with crude suspensions of chloroplasts and those obtained by Hill and others using washed suspensions of chloroplasts is that whereas in the latter case oxygen is liberated, in the former it is taken up. Noack[8]) has also shown that suspensions of the green sediments from crushed leaves take up oxygen during illumination whilst Kautsky and Davidshöfer[5]) ob-

served that grana suspensions from *Clematis*, containing sugar, took up more oxygen during illumination than when in the dark.

The increase in uptake of oxygen during the period of illumination in the present experiments, calculated on a dry weight basis, amounts to five times that observed in Kautsky's experiments with *Clematis* material. This is most probably due to the higher intensity of illumination resulting from the arc light used in the present experiments as compared with the illumination from the 75 watt incandescent lamp used in Kautsky's experiments.

It seemed probable that an investigation of the effect of HCN on the uptake of oxygen by these suspensions might give an indication of the nature of the enzyme systems involved in these processes.

The experiment was carried out in three parts: in each case the uptake of oxygen by 8 ml of the crude chloroplast suspension, obtained from the same plant material as used in the previous experiment, was measured first during a period of darkness, followed by a period of illumination. In the second part of the experiment no additions were made to the chloroplast suspension. In the first part of the experiment 1 ml of 0.02 mol. KCN solution, neutralised to pH 7.0, was added to the chloroplast suspension at the commencement of the experiment, the suspension being in the dark. In the third part of the experiment 1 ml of 0.04 mol. KCN was added to the chloroplast suspension whilst the suspension was still in the dark but sixty minutes after the commencement of the experiment.

The results of these experiments are summarized in table II and are represented graphically in fig. 2.

Uptake of oxygen in the dark is not affected by KCN at a concentration of 0.0023 mol. The somewhat higher rate of uptake of oxygen during the dark period in experiment III is not due to the HCN as the KCN was added 60 mins. after commencement of the experiment and did not cause a change in the slope of the curve.

In all three experiments there was a considerable increase in uptake of oxygen as soon as illumination commenced and it is beyond doubt that under these circumstances the addition of the lower concentration of KCN accelerated the uptake of oxygen. On the other hand the reverse is the case when the higher concentration of KCN was added; the difference between the rate of uptake of oxygen in the dark period and during the period of illumination being 52 mm^3 per hour as compared with a difference of 86 mm^3 per hour i.e. a

TABLE II

The influence of HCN on the uptake of oxygen by crude chloroplast suspensions (mean uptake of oxygen in mm³ per hour)

No. of experiment	Uptake of oxygen whilst in the dark	Uptake of oxygen during illumin.	Ratio of uptake of oxygen in light to uptake of oxy. in dark	Increase of rate of uptake of oxygen in the light as compared with uptake of oxygen in the dark expressed as a %age of the increase in the control experiment
II. Control, no additions	41	127	3.1	—
I. 1 ml of 0.02 mol. KCN added at beginning of exp. final concn. of KCN 0.0023 mol.	40	251	6.3	+146%
III. 1 ml of 0.04 mol. KCN added 30 mins. after commencement of exp. final concn. of KCN 0.0044 mol.	49	101	2.1	— 40%

decrease of 40% compared with the difference in the control experiment.

The main respiration processes of intact cells are completely inhibited by 0.001 mol. HCN [9]) (p. 258). Since the main respiration processes are associated with an intact cell structure they are completely eliminated when the leaf is crushed. The remaining accessory respiration processes can be separated from the intact cell and are not affected by HCN. From such considerations it may be expected that, as was in fact observed, the respiration of the chloroplast suspension in the dark would not be affected by the presence of 0.004 mol. HCN.

The considerable increase in the rate of uptake of oxygen during the period of illumination in the presence of 0.0022 mol. HCN is less easily understood. Various authors have recorded a stimulation of respiration in the cells of intact plants when in the presence of HCN. Van der Paauw [10]) showed that at a concentration of KCN of 0.0001 mol. the respiration rate of both *Hormidium flaccidum* and

Stichococcus bacillaris was considerably increased, and in the case of the latter organism to the extent of 153% when the concentration of HCN was 0.005 mol. Warburg [15]) observed an increase in the respiration rate of starved *Chlorella* cells when placed in the presence of comparatively high concentrations of HCN, the respiration rate only decreased markedly when the concentration of HCN reached 0.1 mol. It is well known that the "old" yellow enzyme of Warburg

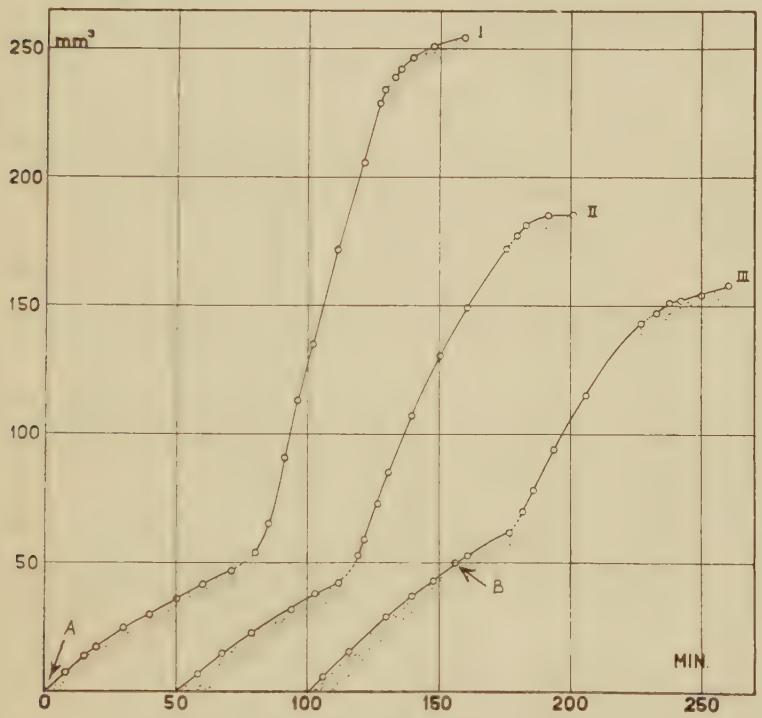


Fig. 2. The influence of HCN on uptake of oxygen.

II. Curve of uptake of oxygen in the absence of HCN.

I. Curve of uptake of oxygen when HCN (total concn. 0.0022 mol) is added during the dark period at *A*. There is no influence on rate of uptake of oxygen whilst the suspension is in the dark but rate of uptake of oxygen is increased when the suspension is subsequently illuminated.

III. Curve of uptake of oxygen when HCN (total concn. 0.0046 mol.) is added during the dark period at *B*. There is no influence on the rate of uptake of oxygen during the dark period but the rate of uptake of oxygen decreases when the suspension is subsequently illuminated. (Dark periods shadowed).

may play an important role in residual respiration and that the activity of this enzyme is increased in the presence of low concentrations of HCN. The question may therefore be raised as to whether or not the flavo protein enzymes play a part in the photo oxidative processes involved in the present experiments.

It seemed of interest in this connection to investigate the effect of addition of glucose to the chloroplast suspension. Glucose is the principal hydrogen donator in the main respiration processes susceptible to HCN. The increased respiration rate of *Chlorella* in the presence of added glucose is easily inhibited by HCN according to Emerson[1]). Since in the present experiments the uptake of oxygen in the dark was shown to be unaffected by HCN it might be expected that the addition of glucose would not affect the rate of uptake of oxygen. That this proved to be the case can be seen from the results in table III and the corresponding graph in fig. 3.

TABLE III

The effect of glucose on the uptake of oxygen by crude suspensions of chloroplasts. (Mean uptake of oxygen in mm^3 per hour).

Experiment	Time in mins.	Uptake of oxygen in the dark	Uptake of oxygen during illumination
I. Glucose (20 mg) added during illumination (see fig. 3)			
a. Dark period before glucose was added	0–47	78	—
b. Illumination period before glucose was added	47—88	—	175
c. Illumination period after addition of glucose	88–129	—	180
II. Glucose (85 mg) added during the second dark period			
a. First dark period before glucose was added	0–75	55	—
b. First illumination period before glucose was added	75–138	—	130
c. Second dark period before glucose was added	138–168	5–1	—
d. Second dark period after addition of glucose	168–198	1–0	—
e. Second illumination period	198–220	—	120
III. Glucose (85 mg) added during dark period. The leaves had previously been kept in dark for two days. (See fig. 4)			
a. Dark period before addition of glucose	0–52	43	
b. Dark period after addition of glucose	52–115	34	

Experimental Material used. 1,5 g of *Avena* leaf material from plants grown in a complete waterculture from 28–2 to 15–5, were crushed and the chloroplasts suspended in 22.5 ml salt solution (as above, without $NaHCO_3$). 8 ml of this suspension were placed in a Warburg flask, air above the suspension.

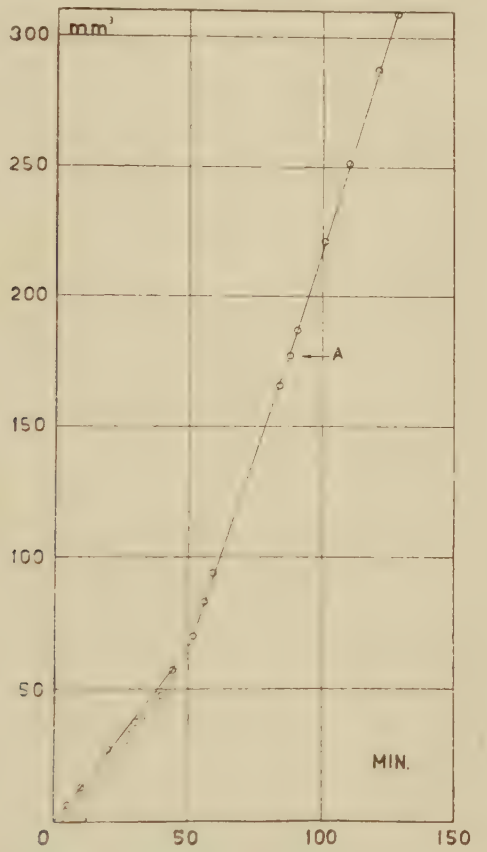


Fig. 3. The influence of glucose on uptake of oxygen.
Addition of glucose does not appear to have any influence on the rate of uptake of oxygen by the suspension.

Experiment I shows that addition of glucose during the period of illumination does not markedly increase the rate of uptake of oxygen, the small increase of 5 mm^3 per hour not being significant. This state of affairs may have been brought about by the presence of adequate supplies of carbohydrate from the leaf itself. In order to

exclude this possibility the leaves from which the crude suspensions of chloroplasts were obtained for the third experiment were covered for two days by strips of white paper. the side of the paper in contact with the leaf being covered with black paper, in order to prevent carbon assimilation. The rate of uptake of oxygen during the dark period by the crude suspensions of chloroplasts from leaves treated in this way was much less than for the corresponding period in the other experiments and the addition of glucose did not alter this lower rate of uptake of oxygen.

In the second experiment glucose was not added until some time during the second dark period i.e. not until after the material involved in the uptake of oxygen in the dark had been exhausted. The rate of uptake of oxygen during the second dark period was initially 5 mm^3 per hour before glucose was added falling to 1 mm^3 per hour compared with the 55 mm^3 per hour in the first dark period. Addition of glucose during the second dark period did not alter the rate of uptake of oxygen whereas in the second illumination period which followed, the uptake of oxygen amounted to 77% of that in the first illumination period.

From these experiments it may be concluded that these crude suspensions of chloroplasts are completely unable to oxidise glucose.

According to Oppenheimer (9, p. 260) the accessory respiration system which remains after cell structure is destroyed is often able to utilise amino acids as substrates. It is to be expected that plants possess a mechanism by which the production of energy necessary for the maintenance of life can be achieved when carbohydrate reserves are exhausted. Although proteins will be among the first materials to be considered in this connection the energy liberated during the breakdown of proteins to amino acids is too small to be of any consequence. The energy liberated during the decarboxylation and deamination of amino acids is, however, much greater and it may well be that such processes provide secondary supplies of energy. Spoehr and McGee[14]) demonstrated that the addition of glycocoll and asparagine caused a considerable increase in the respiration of intact *Helianthus* leaves. Therefore, it seemed of interest to investigate the effect of a substance such as asparagine on these crude suspensions of chloroplasts especially since this substance occurs in many plant tissues.

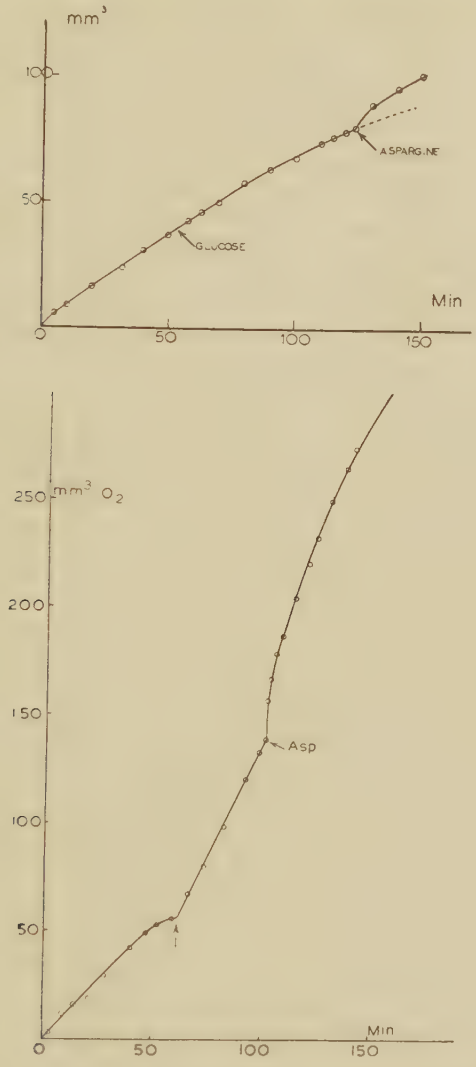


Fig. 4. The influence of asparagine on uptake of oxygen.

a. Curve shows that there is no increase in the rate of uptake of oxygen by the suspension when glucose is added during the dark period. There is a slight increase in the uptake of oxygen by the suspension when asparagine is added.

b. Curve showing the increase in the rate of uptake of oxygen by the suspension when asparagine is added whilst the suspension is being illuminated. The increase is many times the increase observed when asparagine is added during the dark period (fig. 4*a*).

Experimental Material used. 1.5 g of *Avena* leaf material (from plants grown in a complete water culture from 28–2 to 16–5) were crushed and the chloroplast preparation suspended in 22.5 ml of salt solution (as above, without $NaHCO_3$). 8 ml. of this suspension were placed in a Warburg flask.

In the first experiment 10 mg of asparagine was added to the suspension during the course of the dark period. In this case the leaves had been kept in the dark for two days. On comparing the rate of uptake of oxygen during the 30 minutes of the dark period immediately prior to the addition of the asparagine with the rate of uptake of oxygen during the 30 minutes of the dark period immediately following the addition of the asparagine it will be observed (Fig. 4*a*) that there is an increase of 53% in the quantity of oxygen taken up by the suspension i.e. mean uptake of oxygen increases from 5 mm^3 in ten minutes to 7.7 mm^3 in ten minutes.

The effect of adding asparagine to the chloroplast suspension during illumination was investigated in the following two experiments; the results of the first experiment are summarised in table IV.

TABLE IV

Uptake of oxygen by crude suspension of chloroplasts before and after the addition of asparagine (10 mg) in 0.5 ml water during the period of illumination

	Time in mins.	Rate of uptake of oxygen in mm^3 per min. during successive 10 mins. periods	Mean rate of uptake of oxygen in mm^3 per 10 mins.
Dark period	10	—	
	20	11	
	30	10	9.2
	40	10	
	50	9	
	60	5	
Illumination period	61	—	
	70	22	
	80	21	21.3
	90	21	
	100	21	
	101 — 10 mg asparagine added	—	
	110	54	40
	120	31	
	130	25	

The data of a second experiment are graphically represented in fig. 4*b* and it will be observed that the results are exactly the same. In the latter case the rate of uptake of oxygen during that part of the illumination period before the addition of asparagine was 21.5 mm^3 per 10 mins. whilst during the 10 mins. of the illumination period immediately after the addition of asparagine the rate of uptake of oxygen increased to 57 mm^3 per 10 mins.

It seems, therefore, that although cell respiration processes catalysed by iron containing enzymes are absent from these crude suspensions of chloroplasts there remains a considerable respiration system which is not inhibited by HCN and which is able to utilise an amino acid as a substrate during the dark period.

The increase in uptake of oxygen after the addition of asparagine during the illumination period amounts to 32.7 and 33.5 mm^3 respectively in the first ten minutes. This points to increased accessory respiration processes under the influence of illumination or to increased photo oxidation resulting from the presence of an easily oxidisable substrate. If the latter alternative is the case, manganese may play a role in the process since it has been shown in a previous paper that this heavy metal promotes the development of high redoxpotentials during illumination in the presence of oxygen. The possibility of this relationship is supported by the observations that manganese activated enzyme processes, as well as the uptake of oxygen by crude suspensions of chloroplasts, are not inhibited by rather high concentrations of HCN(9, p. 77). It therefore appeared to be desirable to investigate the effect of added manganese on the uptake of oxygen by a crude suspension of chloroplasts.

Experimental Material used. Chloroplast material from 1 g of *Avena* leaf material (from plants grown in the greenhouse in complete water culture solution from 18–8 to 27–10) was suspended in 15 ml water.

8 ml of the suspension were placed in a Warburg flask containing air above the suspension; 0.2 mg. Manganese was added as $MnSO_4$ dissolved in 0.5 ml water.

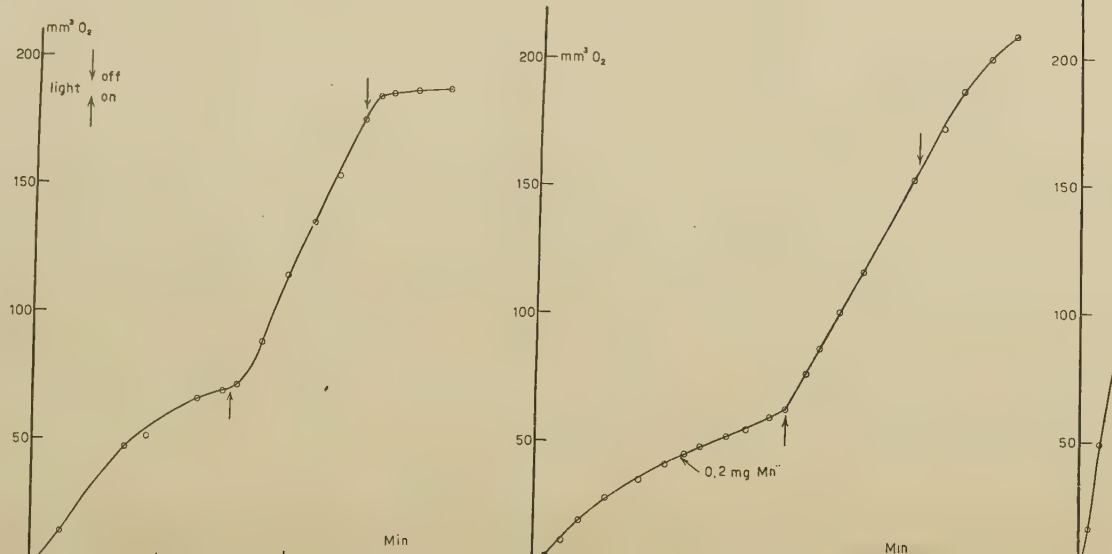
In the first experiment the manganese was added during the dark period and, as can be seen from fig. 5*b*, it had no influence on the rate of uptake of oxygen by the suspension there being no change in the slope of the curve after the addition of manganese.

Fig. 5. The influence of manganese on uptake of oxygen.

a. Curve showing uptake of oxygen by an untreated suspension. The curve indicates that towards the end of the dark period residual respiration decreases because of exhaustion of cellular reserve material. When the suspension is illuminated the rate of uptake of oxygen increases 2–3 fold. During the second dark period the residual respiration quickly decreases to a negligible amount.

b. Curve showing that when manganese is added during the dark period the rate of uptake of oxygen by the suspension is not noticeably affected. During the second dark period the uptake of oxygen continues at the same rate as during the preceding period of illumination, thereby indicating that the higher respiration rate of the illumination period is maintained during the second dark period (see text).

c. Curve showing that when manganese is added during the illumination period, the rate of uptake of oxygen increases. In this case also there is no rapid decrease in the rate of uptake of oxygen during the dark period following illumination.



The results of two experiments in which manganese was added during the illumination period are shown in the following table.

TABLE V

The influence of manganese ions on the uptake of oxygen by crude suspensions of chloroplasts

	Time in mins.	Cumulative uptake of oxygen in mm^3 from beginning of experiment.	Mean uptake of oxygen in mm^3 per minute in successive 5 minute periods *)
	4	20	—
	9	56	5.2
	14	83	6.8
	18	103	6.0
	22	129	5.6
	26	143	4.2
Illumination-period	30	156	3.2
	31 — 0.2 mg Mn as $MnSO_4$ added		
	34	189	7.2
	38	203	4.0
	42	216	3.0
	46	228	2.8
	50	238	2.2
2nd experiment using the same material (Fig. 5c).			
	4	16	4.0
	8	49	7.4
	12	69	5.6
	16	84	4.2
	20	108	3.4
	24	211	2.8
	28	132	
	30	139	
Illumination-period	31 — 0.2 mg Mn added as $MnSO_4$		
	34	168	7.2
	38	189	4.6
	42	206	3.6
	46	227	3.0
	54	242	2.8
	60	259	2.8
	64	264	2.4
	68	279	1.8
Dark	72	285	1.2
	76	290	1.0

*) As the periods between the observations were somewhat irrigular, these data have been derived from the curves.

When manganese was added during the illumination period the uptake of oxygen by the suspensions usually increased as shown by the 125% and 157% increases respectively in the case of the two experiments the results of which are set out in Table V. These increases in rate of uptake of oxygen only lasted for a short time i.e. 10–20 minutes. In a few other cases the addition of manganese produced only a negligible effect; the cause of this could not be ascertained. The increased uptake of oxygen following the addition of manganese is in agreement with the specific role of manganese in oxygen transfer and oxidation reduction reactions occurring during photosynthesis; this was pointed out in the previous paper.

The absence of any effect, resulting from the addition of manganese to the chloroplast suspension during the first dark period, on the uptake of oxygen was to be expected as the redoxpotential is also not influenced under similar conditions.

A suspension to which manganese has been added also differs in another respect from a suspension to which manganese has not been added. In the case of the suspension without added manganese the rate of uptake of oxygen soon slows down to a negligible amount (fig. 5*a*) after the end of the illumination period; whereas in those cases where manganese has been added to the suspension the rate of uptake of oxygen continues at the same level for some time after the end of the illumination period or the decrease in the rate of uptake of oxygen is much less than in the absence of manganese (fig. 5*b* and *c*).

In the previous paper it was shown that chloroplast suspensions to which manganese had been added were still able to form peroxide whilst in the dark following a period of illumination. The phenomenon of prolonged uptake of oxygen in the dark period following a period of illumination may perhaps be ascribed to the same cause and might also be regarded as further evidence for the formation of a more or less stable intermediary HX compound.

Discussion. Since the activity of the system which brings about the uptake of oxygen during the dark period is not inhibited by relatively high concentrations of HCN, and the fact that this respiratory system, acting during the dark period, appears to be able to utilise amino acids as a substrate whereas glucose as such cannot be used, indicates that we are dealing with an accessory respiratory system which survives the mechanical destruction of cellular structure.

During the period of illumination the rate of uptake of oxygen increases as might be expected from the previously observed rapid increase in redoxpotential under these conditions. Since glucose is not dehydrogenated under such conditions the system involved is more than one of simple photo oxidation. This observation together with the fact that addition of asparagine during the illumination period causes an increase in the rate of uptake of oxygen many times greater than the increase in the rate of uptake of oxygen brought about when asparagine is added during the dark period make it highly probable that, in spite of the destruction of cell structure, the reaction remains enzymatic in nature and highly specific. The enzyme in question may be an amino acid oxidase [17]) of the yellow enzyme group. The observation that uptake of oxygen increases markedly on addition of moderate quantities of HCN also points in this direction. According to Oppenheimer c.s. [9]) (p.261) oxygen transfer via "oxytropic" dehydrogenases and the yellow enzyme is accentuated in the presence of HCN. In connection with the high redoxpotentials which were observed, and which led to the conclusion that hydrogen peroxide was formed, it is interesting to note that according to the same authors, oxygen is not completely reduced to water during the regeneration of the reduced leuco form of the yellow enzyme but only as far as hydrogen peroxide.The highly specific nature of the reactions makes it rather improbable that either the H atoms or the OH radicals resulting from the photo oxidation of water, during the first stage of photosyntheses, will have even a short period of free existence in which to react vigorously with many kinds of organic substances. Such, presumably harmful, reactions can only be avoided if these radicals are instantaneously incorporated in the carriers or enzymes by which they are produced.

There seems no reason to suppose that these reactions e.g. the photo oxidation of amino acids, cannot take place in intact leaves where they will be kept within bounds by the cell structure and the limitation this places on the diffusion of oxygen into the cell.

Moreover, it should be noted that rather high light intensities, much higher than are necessary for normal photosynthesis, were used in these experiments. The experiments of Myers and Burr [6]) are of great interest in this connection since they observed that oxygen liberation by intact *Chlorella* cells is turned to uptake of oxygen when the light intensity is increased from 4,000 to 27,700 foot cand-

les. Further they showed that, at high light intensities, the final rate of photo auto oxidation by intact *Chlorella cells is unaffected by the addition of cyanide* which demonstrates that notwithstanding the presence of iron (haemin) containing enzymes in the cells, these enzymes do not take part in the light induced reactions.

This makes rather highly probable the assumption that the photo oxidation processes examined *in vitro* in the present experiments and the photo auto oxidation reactions occurring *in vivo* are closely related and even attributable to the same cause.

The addition of manganese to the crude suspensions of chloroplasts did not alter the rate of the residual respiration during the dark period; this is in agreement with the observation that under these circumstances the redoxpotential also remains unchanged. In a number of cases a decided increase in the uptake of oxygen was observed directly after the addition of manganese during the illumination period, this lasted only a short time and was not observed in every case. This is most probably brought about by the exhaustion of cellular reserve materials. It will be seen from table V that the mean rate of uptake of oxygen in successive periods was already diminishing before the manganese was added and had been reduced by more than 50%, from 6–8 mm^3 to 3.2 mm^3 per minute after 30 minutes. Immediately after the addition of manganese it increases from 3.2 mm^3 to 7.2 mm^3 per minute and then drops to 2.2 mm^3 per minute within 20 minutes.

The quantity of oxygen taken up per unit time does not depend only on the intensity of the oxidation process but also on the amount of cellular reserve substance present. Since the rate at which these substances are oxidised is increased by the addition of manganese they will disappear sooner from the suspension than they would in the absence of added manganese.

In the light of these experiments the chlorosis observed by many authors [3] [12] [13] in plants which contain an excess of manganese in comparison with their iron content, becomes more intelligible. It is a well known fact that proteins protect chlorophyll from destruction by intense light [11] (p. 537). As the quantity of amino acids in the chloroplast suspensions does not appear to be sufficient to keep photo oxidation going for more than one hour at the high rate observed in these experiments it must be assumed that in vivo amino acids are liberated from proteins or that the proteins themselves can be photo

oxidised under these circumstances. In this way light energy would be dissipated and the chlorophyll protected from bleaching so long as the cellular reserve materials of a protein nature remain unexhausted.

Since an excess of manganese intensifies photo oxidation it is to be expected that the point at which the chloroplasts are no longer protected will be reached much sooner than when only the normal amount of manganese is present.

Chlorosis caused by excess of manganese will not be due to insufficient production of chlorophyll but to photo oxidation of protecting substances promoted by the high redox potentials which in turn are engendered by the insufficient counterbalancing effect of iron, with the result that the chlorophyll is bleached. Further experiments are necessary before this hypothesis can be accepted.

Finally the question may be raised as to whether the balance between photosynthesis and respiration is dependent on light intensity. The complete system in the leaf is more complicated since in addition to normal respiration there is also, during illumination, photo auto oxidation and the rate of this latter process depends largely on the manganese iron ratio and light intensity. At high light intensities, and especially when the manganese iron ratio is also high, the equilibrium will be shifted progressively towards photo oxidation not only because chlorophyll will be bleached and rendered inactive but also because the protein like substances, which are indispensable for photosynthetic activity, are photo oxidised.

The assumption of R a b i n o w i t c h [11]) (page 536) that the inhibition of photosynthesis by excess of light is essentially the same phenomenon as the inhibition by excess of oxygen is not supported by the present author. In the case of excess of oxygen the reducing activity of the HX compound is directed from the CO_2 complex to oxygen, resulting primarily in the formation of hydrogen peroxide, and secondary in the oxidation of cell constituents. (See also the previous paper page 190).

In the case where photosynthesis is inhibited by excess light the photosynthetic system is progressively destroyed by photo auto oxidation in the manner put forward above.

Summary

1. Crude suspensions of chloroplasts show a distinct accessory respiration in the dark.

2. During illumination of such suspensions evolution of oxygen is not observed, on the contrary the uptake of oxygen increases 2–3 fold.

3. The accessory respiration of crude suspensions of chloroplasts in the dark is not inhibited by HCN (up to 0.04%). The uptake of oxygen during the illumination period is increased by a moderate HCN concentration (0.02%) and retarded somewhat by higher concentrations (> 0.04%).

4. The uptake of oxygen is not affected by the addition of glucose either during the dark period or during illumination.

5. The uptake of oxygen is increased by the addition of asparagine both in the dark and during illumination. The increase in uptake of oxygen on addition of asparagine during the period of illumination may exceed considerably the increase which occurs on addition of asparagine during the dark period.

6. The addition of manganese does not affect the rate of uptake of oxygen during the dark period, when added during the illumination period the uptake of oxygen is often temporarily markedly increased.

7. Chlorosis brought about by a too high manganese iron ratio is assumed to be caused by photo oxidation of chlorophyll which is a secondary effect of the rapid destruction of the protective protein substances in the presence of extremely high redox potentials.

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REFERENCES

1) Emerson, R. L., The effect of certain respiratory inhibitors on the respiration of chlorella. J. Gen. Physiol. **10**, 469–477 (1927).
2) Gerretsen, F. C., Manganese in relation to photosynthesis II. Plant and Soil **2**, 159–193 (1950).
3) Gile, P. L., Chlorosis of Pineapple, induced by manganese and carbonate of lime. Science **44**, 855–857 (1916).
4) Hill, R., Oxygen evolved by isolated chloroplasts. Nature **139**, 811 (1937).
5) Kautsky, H., Chlorophyllfluorenszenz und Kohlensäure Assimilation VII. Die Photooxydation. Biochem. Z. **291**, 285, 311 (1937).
6) Myers, J. and Burr, G. C., J. Gen. Physiol. **24**, 45,(1940).
7) Münzer, E., and Neumann, W., Zur Verwendung des Differential Apparatus von Barcroft. Biochem. Z. **81**, 319–331 (1918).
8) Noack, K., Wirkungen des Chlorophylls. Naturwissenschaften **14**, 383 (1926).
9) Oppenheimer, C. and Stern, K. C., Biological Oxidation. The Hague 1939.
10) Paauw, F. van der, The indirect action of external factors on Photosynthesis. Rec. trav. botan. néerland. **29**, 500–620 (1932).
11) Rabinowitch, E. I., Photosynthesis. I. New York 1945.

12) Scholz, W., Über die Chlorose der blauen Lupine und Serradella in ihrer Beziehung zum Eisen und Mangan. Z. Pflanzenernähr A **35**, 88–101 (1934).
13) Somers, I. I. and Shive, J. W., The iron manganese relation in plant metabolism. Plant Physiol. **17**, 582–602 (1942).
14) Spoehr, H. A. and McGee, J. M., Studies in Plant Respiration and Photosynthesis. Carnegie Institute of Washington, Publ. No. 325, 1923.
15) Umbreit, W. W., Burri R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism. Minneapolis 1949.
16) Warburg, O., Über die Geschwindigkeit der photochemischen Kohlensäure Zersetzung in lebenden Zellen. Biochem. Z. **100**, 000 (1919).
17) Warburg, O. and Christian, W., Bemerkungen über gelbe Fermente. Biochem. Z. **298**, 368 (1938).